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Ecodynamique des éléments traces et caractérisation de l'exposition des sols contaminés:
expérimentation et modélisation par les Réseaux de Neurones Artificiels

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Introduction générale

1.Contexte

Le sol est situé à l'interface entre la lithosphère, l'atmosphère, l'hydrosphère et la biosphère. Il est le lieu de transfert et de stockage de matières transitant entre ces compartiments (Gobat *et al.*, 2003).

Cette interface assure également de nombreuses fonctions économiques, écologiques et biologiques. Le sol est le support de nombreuses activités humaines (industrie, urbanisation etagriculture). Le sol constitue une entité vivante et dynamique, en perpétuelle évolution, pouvant être perturbée par de nombreux facteurs; parmi ces derniers, le développement des activités humaines et spécialement les activités industrielles accompagnées du rejet de nombreuses substances chimiques qui ont parfois fortement altéré la qualité des écosystèmes terrestres. Les principales problématiques associées à ces rejets sont l'**eutrophisation** (apports excessifs de nutriments) et la **contamination** (apports de substances chimiques potentiellement toxiques). En particulier la compréhension du transfert, de l'accumulation et du devenir des éléments traces (ET) comme les métaux et métalloïdes d'origine anthropique dans le milieu naturel est nécessaire pour évaluer les risques pour les écosystèmes et la santé de l'homme. En effet la connaissance de la forme physique et chimique des métaux, d'origine naturelle ou anthropique, est primordiale pour comprendre les mécanismes de transfert dans le milieu naturel et l'accumulation par les organismes vivants. Le développement des sociétés a en effet conduit à une utilisation extensive et intensive des ET mais également à une pollution généralisée à grande échelle. Contrairement aux composés organiques qui peuvent être ramenés à leurs éléments de base par combustion, les ET ne s'éliminent pas; ils changent de forme chimique et sont toujours susceptibles de remobilisation dans l'environnement par des mécanismes de transformation naturels.

Les éléments traces potentiellement toxiques (PTTE) tels que le Cuivre (Cu), le Zinc (Zn), le Chrome (Cr), le Molybdène (Mo) et l'Arsenic (As) sont un des principaux polluants générés par les activités industrielles. Les concentrations excessives de ces éléments traces potentiellement toxiques (PTTE) dans les sols induisent des risques importants pour la santé humaine, la santé animale et végétale. Ils peuvent également causer des perturbations majeures sur les écosystèmes terrestres (Alloway, 1995; Kabata-Pendias et Pendias, 1992; Ross, 1994). En raison de la forte persistance des

éléments traces potentiellement toxiques (PTTE) dans les sols contaminés, la difficulté de gestion de leur transfert, accumulation, devenir et toxicité potentielle, constituent un problème environnemental qui nécessite une solution efficace et durable.

2.Contexte scientifique

Dans un contexte d'évaluation des risques écologiques, de gestion des milieux naturels et de développement durable, il est capital d'acquérir des connaissances sur le devenir des PTTE dans ces différents compartiments abiotiques et biotiques et de définir les mécanismes qui conditionnent leurs transferts, leurs capacités de bioaccumulation et, *in fine*, leurs effets toxiques et écotoxicologiques sur les différents niveaux biologiques d'intégration. En effet la connaissance de la forme physique et chimique des ET, d'origine naturelle ou anthropique, est primordiale pour comprendre les mécanismes de transfert dans le milieu naturel et d'accumulation par les organismes vivants. La spéciation chimique (état d'oxydation, environnement moléculaire) est un paramètre fondamental qui contrôle la migration, la biodisponibilité et la toxicité des éléments chimiques dans les eaux, les sols et les sédiments. Ce paramètre résulte des interactions entre solutés, surfaces minérales, substances organiques et biologiques.

Les PTTE sont des contaminants prioritaires et résultent d'activité anthropique, industrielle ou agricole. En particulier le sulfate de cuivre et le CCA (arséniate de cuivre chromé, Cu, Cr, As) ont été très utilisés pour traiter le bois. Le cuivre servait de fongicide et As d'insecticide. En France, la base BASOL mentionne au moins 23 sites largement pollués en As, Cu et Cr suite à l'utilisation des sulfates de cuivr et du CCA. Le devenir des 3 éléments montre des contaminations d'eaux souterraines (140-450 µg As/L, sites du département de la Loire) et de sols à proximité des aires d'autoclavage, d'égouttage et de stockage des bois (Rayzal et Deroubaix, 1998). La contamination du sol atteint 300-11 000 mg As/kg et plus de 12 000 mg Cu/kg. De pluse danger des faibles doses d'As rencontrées en cas de contamination anthropique a été réévalué à la hausse, notamment pour la santé humaine (Laperche *et al.*, 2003). Utiliser du CCA pour traiter des bois à usage domestique est prohibé aux USA depuis décembre 2003. Le guide BRGM établit la connaissance insuffisante de l'écodynamique de ces 3 ET (éléments traces) (Laperche *et al*., 2003). Le seul suivi de la teneur totale du sol renseigne le type de danger, mais n'évalue pas les risques. Par contre, le suivi simultané de l'écodynamique et des effets écotoxicologiques de ces 3 contaminants sur un site instrumenté présente plusieurs intérêts. Les impacts écotoxicologiques des ET dépendent de leur devenir dans l'écosystème, de leur spéciation lors de l'exposition et de l'imprégnation, des interactions potentielles (synergie, additivité, antagonisme) et des récepteurs. Ainsi les fiches toxicologiques et environnementales pour As, Cr, Cu et leurs dérivés

inorganiques (Pichard *et al*., 2003, 2004) signalent par exemple une quantification insuffisante du transfert des arséniates (As^{+V}) séparé de celui des arsénites (As^{+III}) du sol aux végétaux; elles déplorent que les facteurs de bioconcentration se rapportent à As total. La co-évolution des différentes espèces chimiques est à considérer. Beaucoup d'espèces chimiques de l'As (As^{III}, As^{V}), du Cr (Cr^{III} et Cr^{VI}) et du Cu (Cu^{I} et Cu^{II}), sous la forme d'aqua-ions ou complexés aux composés humiques, sont susceptibles d'être présentes dans les sols contaminés au CCA (Mattusch *et al.*, 2000; Molenat *et al.*, 2000). Toutes n'ont pas la même toxicité. La spéciation des contaminants est donc nécessaire. En plus, la connaissance des interactions chimiques entre les solutés et les phases minérales ou organiques repose en général sur des mesures en conditions différentes de l'*in-situ* ou des phases peu réactives face à l'activité biologique, qui peuvent fausser les modèles de biodisponibilité et ne considèrent pas l'aspect cinétique notamment de l'échange sol-solution. Une évaluation détaillée des effets écotoxiques dus au bois traité CCA existe pour les milieux aquatiques (Brooks, 2003), mais à notre connaissance, le devenir en milieu terrestre des 3 ET du CCA (dont la caractérisation de la spéciation et processus réactionnels, en cinétique, le devenir des colloïdes), les facteurs de remobilisation, les impacts écotoxiques et écologiques (notamment en terme de biodiversité) en sites aux sols contaminés en CCA sont insuffisamment renseignés (Matera et le Hecho, 2001; Matera et al 2003). Les processus biogéochimiques intervenant dans la réactivité, le devenir et la toxicité des contaminants sont peu documentés en situation de mobilisation ou d'atténuation avec développement de communautés végétales.

Un certain nombre de techniques ont été développées pour assainir les sols contaminés en ET telles que l'excavation, le lavage des sols et le tamisage. Toutes ces technologies sont efficaces, mais chères et destructives, et donc pas durables en terme de consommation de matières premières et de production de déchets, mais aussi très coûteuses pour les grands sites pollués (Basta *et al.*, 2004; Raicevic *et al.*, 2005; Dermont et al., 2008). Par ailleurs, d'autres technologies prometteuses, peu agressives, à faible coût même pour les grands sites pollués, ont la capacité de restaurer l'intégrité physique, chimique et les propriétés biologiques des sols contaminés par les PTTE (Raicevic *et al.*, 2005; Kumpiene *et al.*, 2006, 2008).

Une des technologies prometteuses est la *PHYTOREMEDIATION* ; cette technologie regroupe un ensemble de techniques, basées sur l'utilisation des plantes pour la remédiation des sites contaminés. Elles se définissent comme étant l'utilisation de plantes pour éliminer ou rendre moins mobiles et moins toxiques les contaminants environnementaux. Elles peuvent être appliquées à des contaminants organiques ou inorganiques. Parmi ces techniques, ***la phytostabilisation***, cette technologie est basée sur l'utilisation de plantes afin d'empêcher la dispersion des contaminants dans les sols, les eaux de surface

ou souterraines. Les plantes peuvent immobiliser les contaminants dans la zone racinaire par l'exsudation de composés chimiques, par l'inhibition des protéines de transport dans les membranes racinaires et par le stockage des contaminants dans les vacuoles des cellules racinaires (Bert et Deram, 1999 ; Anonyme, 2009). La phytostabilisation peut être appliquée seule ou en combinaison avec des agents stabilisant *in-situ* (phytostabilisation assistée par des amendements organiques ou minéraux). Ces amendements peuvent améliorer l'efficacité de la phytostabilisation et la production de matières premières à base de plantes par diminution de la mobilité et de la biodisponibilité des ET (Kumpiene *et al.*, 2008; Mench *et al.*, 2010).

La deuxième technologie utilisée pour remédier les sols contaminés en ET est ***la phytoextraction***. Cette technique utilise des plantes afin de prélever les ET des sols contaminés. Ces contaminants vont s'accumuler dans les parties aériennes de la plante via la lignification et dans les vacuoles des cellules de feuille. Aprés l'accumulation des contaminants dans les plantes celle-ci seront ensuite récoltées, les tissus végétaux ayant concentré les ET seront traités par séchage, incinération ou compostage (Mench *et al.*, 2009; Vangronsveld *et al.*, 2009; Zhao and McGrath, 2009). Cette technique peut être améliorée en ajoutant des amendements au sol (phytoextraction aidée) pour rendre les contaminants plus solubles et donc plus disponibles pour les plantes (Prasad et Freitas, 2003; Peng *et al.*, 2005).

3.Les objectifs de la thèse

L'objectif général de cette thèse est d'étudier expérimentalement et par modélisation à l'aide des réseaux de neurones artificiels l'écodynamique des ET dans les sols, les changements résultant pour l'écotoxicité du sol et le fonctionnement de l'écosystème. Il s'agit également d'étudier leur atténuation dans les sols via la phytorémédiation. L'efficacité de plusieurs méthodes de phytoremediation a été évaluée comme la phytostabilisation assistée par des amendements organiques frais ou matures, ou des amendements organiques et minéraux et la phytoextraction assistée par des amendements organiques et minéraux. Les mesures effectuées lors de la phase expérimentale ont été utilisées pour alimenter et construire un certains nombre de modèles par réseaux de neurones pour analyser et étudier les réponses des sols combinant différent facteurs tels que le pH, la conductivité électrique, le carbone organique dissous et le type d'amendement appliqué.

Concernant l'étude expérimentale, les deux options de phytorémédiation sont appliquées respectivement sur un sol naturel contaminé fortement en Cu et sur un technosol contaminé fortement en Mo et Cr. Il s'agit d'étudier la mobilité, la (phyto)disponibilité des éléments races dans les sols et d'acquérir également des connaissances sur des mécanismes réactionnels dans le sol et sur les

concentrations et spéciations dans des sources d'exposition, les impacts et la bioaccumulation sur des récepteurs au niveau individuel, dont une chaîne sol- solution du sol- des communautés végétales s'organisant sur site.

Les travaux expérimentaux s'appuient sur deux sites réels instrumentés. Le premier site post-industriel (site de traitement du bois, contaminant majeur : Cu) est situé au sud de Bordeaux (X 1973.15; Y 376.8). Le second site est un technosol développé sur une décharge de co-produits métallurgiques appartenant à Industeel-France (groupe Arcelor Mittal) située sur la commune de Châteauneuf,Loire (site instrumenté de l'ADEME). On se propose d'étudier l'écodynamique de As, Cu et Cr dans le sol d'un site contaminé au sulfate de cuivre et au CCA et des ET sur un site de technosols présentant une multicontamination métallique (Cr, Pb, Zn, Ni, Cu…).

Concernant la seconde partie des travaux, l'objectif a été de développer une modélisation basée sur les réseaux de neurones artificiels (ANN) s'appuyant sur les données expérimentales. Une telle modélisation est bénéfique, (i) pour analyser et interpréter les expériences en laboratoire et les données de terrain, (ii) pour construire un modèle prédictif permettant de prévoir les réponses des sols en fonction de certains facteurs (pH, EC, DOC, amendements) et (iii) identifier des comportements géochimiques des ET. Ces modèles ANN ainsi crée ont été utilisés afin d'acquérir une plus grande compréhension des processus physico-chimique des ET, pour distinguer les facteurs les plus pertinents, et pour étudier l'effet de la variabilité spatial des paramètres du sol sur la variabilité de la disponibilité et de la phytodisponibilité des ET.

La figure 1 illustre les objectifs de la thèse en deux parties; expérimentation et modélisation ainsi que le lien entre elles.

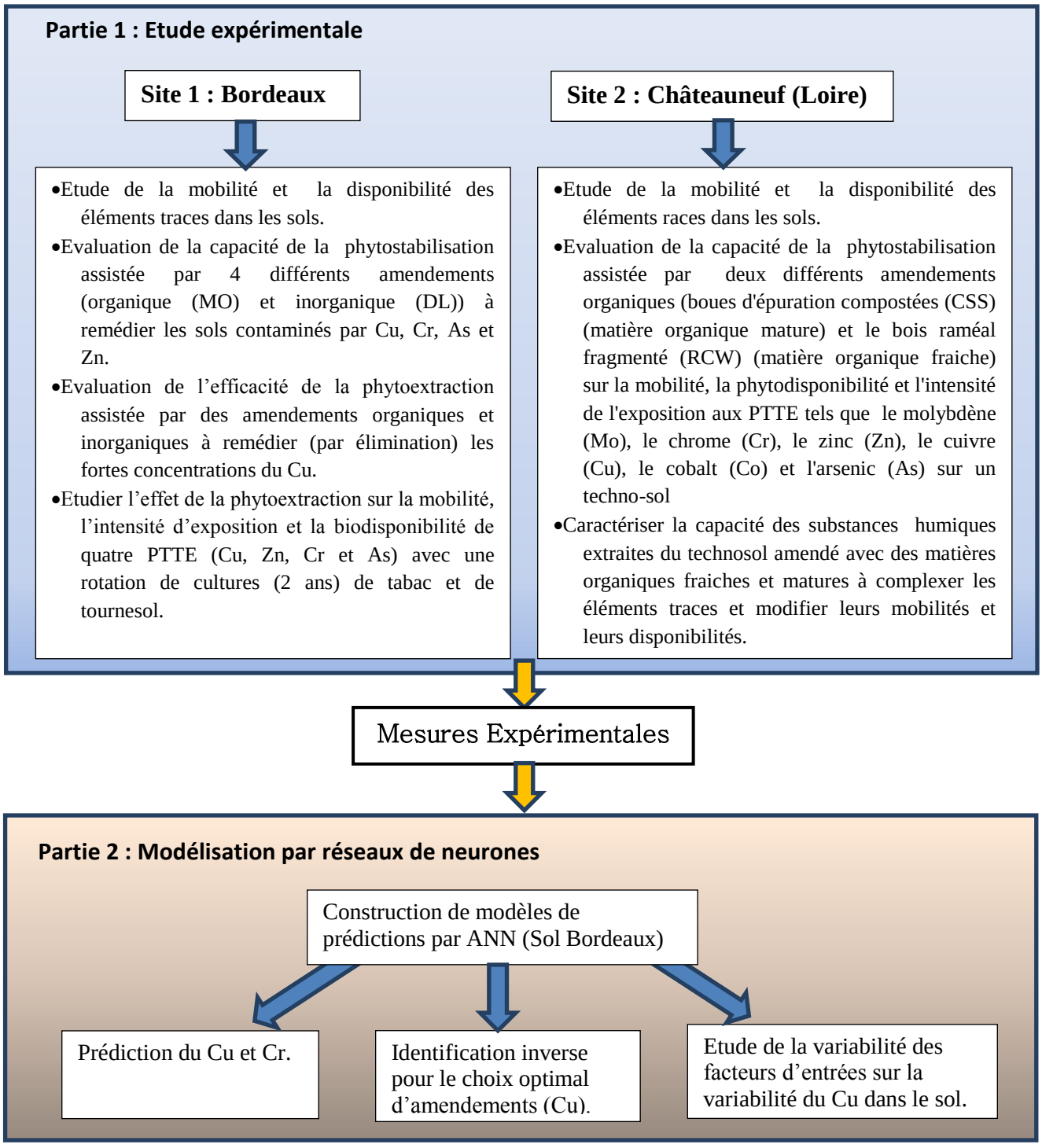


Figure 1. Structure des travaux : Objectifs de la thèse et lien entre la partie 1 (expérimentale) et la partie 2 (modélisation par réseaux de neurones). Les résultats des mesures effectuées en partie 1 ont été utilisés pour construire trois modèles par ANN permettant de prédire quelques comportement des sols étudiés. Notons que les deux parties de la thèse sont complémentaires : la modélisation par ANN ne peut se faire sans les mesures expérimentales et les prédictions par ANN sont indispensables pour l'analyse des expérimentations.

In fine, les objectifs du ce travail s'articulent auteur de deux axes majeurs. Il s'agit:

- de mettre en évidence et de distinguer la technique de phytorémédiation et les amendements les plus efficaces pour remédier les sols contaminés en ET et de comprendre les mécanismes réactionnels dans le sol et leurs conséquences pour les voies d'exposition et les impacts sur les cibles (diversité biologique).
- de mettre en évidence et de tester la capacité d'un modèle par réseaux de neurones artificiels (ANN) considéré comme l'une des techniques majeurs de l'intelligence artificielle à interpréter les résultats obtenus par l'expérimentation et de prédire des réponses à des questions à partir de données expérimentales.

4. Sites d'étude et objectifs spécifiques

La présence d'ET dans les sols génère des questions scientifiques par rapport à leur impact et leur devenir. Elles concernent principalement l'identification des processus et de l'évaluation des options de phytoremedition qui peuvent contrôler la mobilité, la disponibilité et la biodisponibilité de ces éléments dans les sols contaminés en lien avec les conditions du milieu responsables de leurs transferts depuis les sols vers le compartiment biologique.

La connaissance de ces processus est nécessaire pour caractériser le risque que constitue un sol contaminé et définir comment cette contamination peut participer à la dégradation d'un écosystème.

Dans ce travail, les différentes problématiques liées à la présence de sol contaminés en ET et l'évaluation des options de phytoremédiation ont été étudiées sur deux sites :

Le premier site (sol naturel contaminé), est situé dans le sud-ouest de la France, département de la Gironde (44 ° 43'N, 0 ° 30'O). 6 ha de ce site sont constitués précédemment pour stocker les bois traités par des produits chimiques pour les protéger contre les champignons et les bactéries, *etc*….. Le sulfate de cuivre et de créosote ont été utilisé de 1913 à 1980, et l'arséniate de cuivre chromé (CCA standard type C) après 1980. Depuis 1988, le CCA a été seulement utilisée. Ce site est divisé en dix sites, P1 à P10 en fonction des activités passées et présentes, les communautés végétales, le témoignage des employés, des photos aériennes et archivées et l'histoire du site. Le site P3 du sol a montré un manque de matière organique et une concentration de Cu supérieur à de la valeur de seuil, à savoir 35 mg Cu kg^{-1}, justifiant ainsi une forte évaluation de la contamination en Cu (Baize, 1997).

Nos essais sur le terrain ont été effectués sur deux parcelles (150 m^2) de la plate-forme de phytoremédiation BIOGECO. Les deux parcelles ont été établies en 2006 sur le site P3, qui est une

zone abandonnée, autrefois utilisé pour empiler des bois traités et les poteaux électriques, à 400 m de l'usine de traitement du bois. Ces deux parcelles sont divisées à 16 sous parcelles. Sur les 16 sous parcelles, un essai de phytostabilisation assisté a été appliquée pour évaluer la capacité de cette technique à remédier une grand surface fortement contaminé en Cu. Dans les parcelles P3, trois espèces salicacées, une espèce d'arbuste Fabacées, et six espèces de Poaceae ont été plantées. Ces plantes ont été accompagnées par des amendements inorganiques et organiques ; 4 parcelles sont restées sans aucun amendement (UNT), 4 parcelles ont reçues 0,2% de calcaire dolomitique (DL), 4 parcelles ont reçues 5% de compost (OM) et les dernières 4 parcelles ont été traitées avec un mélange de DL et de OM (OMDL).

Le deuxième site (technosol), est le crassier métallurgique d'Industeel-Loire, situé dans le village de Châteauneuf (Rives-de-Gier) entre Saint-Etienne et Givors (France) (44 ° 43'N, 0 ° 30'O), au sud-est de la France. Le site de Châteauneuf s'étend sur 1500 m de long et une moyenne d'environ 200 m de largeur le long de la vallée du Gier, entre le réviere et les contreforts du Massif du Pilat. Le site d'étude a eu beaucoup d'activités industrielles datant de 1845 et ce jusqu'à l'heure actuelle, tels que la production d'acier, *etc*... Ces activités industrielles ont conduit à la contamination du site avec une forte concentration d'éléments métalliques tels que le fer, le manganèse, le plomb et d'autres métaux dans la composition des alliages principaux (Cr, Mo, Al, Ni, Zn, Cu ....). Pour l'étude de la rémédiation de ce site par phytostabilisation assistée, le site est divisé en deux parties. Chacune de ces deux parties est subdivisée en 9 sous parcelles dont 9 sous parcelles *in situ* appelées « parcelles in situ » (P) et 9 sous parcelles de surveillance équipées par des lysimètres appelées « parcelles lysimétriques » (L).

Nos travaux s'appliquent sur les parcelles lysimétrique (L). Ces 9 sous-parcelles lysimétriques (L x W x D = 10 x 5 x 1,5 m^3) ont été installées dans des fosses creusées dans le sol. Ces sols ont été échantillonnés et homogénéisés à l'avance afin de fournir des conditions géochimiques comparables aux parcelles in situ. L'objectif de ces parcelles lysimetriques est de mesurer la quantité d'eau arrivant et sortent de ces parcelles, et la quantité de métaux dans le lixiviat.

Sur ces parcelles lysimétriques un essai de phytostabilisation a été mis en place, assisté par des amendements organiques (frais (RCW) et mature). Les neuf parcelles sont: A) 3 parcelles de sols non amendés (NE) ; B) 3 parcelles de sol amendés avec du bois raméal fragmenté (RCW) et C) 3 parcelles de sols traités avec des boues d'épuration compostées (CSS) ayant une grande maturité. Sur les trois premières parcelles une plante classique à été cultivée, sur la seconde des trois parcelles des plantes métallicoles ont été cultivées et des plantes non-cultivées se sont développées sur les trois dernières parcelles.

5.Structure de la thèse

Ce mémoire est organisé en trois parties distinctes qui exposent les problèmes, et présentent la démarche et les apports scientifiques associées.

La **première partie (synthèse bibliographique)** est subdivisée en trois chapitres. Le **premier chapitre** (1.1) présente la dynamique des ET dans le sol, leurs origines et les facteurs influençant leurs mobilité et disponibilité dans les sols ainsi que leurs réactions avec les constituants organiques et minéraux des sols. Le **deuxième chapitre** (1.2) présente les sites contaminés en ET et les différentes techniques de remédiation des sols contaminés. Dans ce chapitre on va détailler la phytostabilisation et la phytoextraction notamment les avantages et les inconvénients de chaque technique. Le **troisième chapitre** (1.3) introduit la modélisation mathématique dans le domaine des sciences du sol, et les raisons pour lesquelles on a choisit d'applique un modèle correlatif (ANN) dans ce travail.

La **deuxième partie (Résultats par sites)** expose successivement les résultats obtenus pour chacune des problématiques étudiées. Les **chapitres 2.1 et 2.2** exposent respectivement les résultats relatifs à la phytostabilisation (Chapitre 2.1) et à la phytoextraction (chapitre 2.2) assistées par des amendements organiques et minéraux sur un sol naturel contaminé en sulfate de cuivre et au CCA. Le **chapitre 2.3** expose les résultats relatifs à la phytostabilisation assistée sur un technosol fortement contaminé en ET (e.g. Mo et Cr) en utilisant deux types d'amendements organiques (frais et matures). Enfin, le **chapitre 2.4** présente les résultats obtenus sur l'effet des substances humiques extraites du technosol sur la mobilité et la disponibilité des ET dans ce sol.

Enfin, la **troisième partie (Modélisation par les réseaux de neurones artificiels)** présente les résultats obtenus lors de l'application de ce modèle pour interpréter nos résultats expérimentaux et pour prédire des résultats qu'on n'a pas obtenus par l'expérimentation. Cette troisième partie est subdivisée en trois chapitres. Le **chapitre 3.1** expose l'application d'un modèle par réseau de neurones pour la prédiction de la phytodisponibilité du Chrome de sols contaminés phytorémédiés basé sur la prédiction des sorties en fonction d'entrées (ANN direct). Le **chapitre 3.2** expose l'identification inverse des traitements optimaux pour réduire la phytotoxicité du cuivre dans des sols contaminés phytorémédiés basée sur une modélisation par réseaux de neurons. Le **chapitre 3.3** expose l'effet de la variabilité des paramètres chimiques du sol sur la variabilité de la concentration du cuivre phytodisponible dans un sol contaminé phytorémédié à l'aide d'une modélisation hybride par réseaux

de neurones et simulations de Monte Carlo. Ici on a appliqué la modélisation stochastique d'un problème de type entrées-sorties tenant compte de la variabilité statistique (loi normale) des entrées.

1ére partie

Synthèse bibliographique

Cette première partie est conçue pour fournir la base théorique sur la quelle s'est effectué ce travail. Nous fournirons au début de cette partie une définition détaillée des éléments traces, leurs origines dans le sol, leurs effets toxiques sur l'environnement et les facteurs qui influent sur leur mobilité et leur spéciation dans les sols. Ensuite, nous présenterons comment la présence de ces éléments peut générer des contaminations ainsi que des informations sur les sites contaminés par ces éléments en France. Ensuite nous présenterons les techniques de rémédiation des sols contaminés par l'utilisation des plantes (phytorémédiation), en particulier la phytoextraction et la phytostabilisation. A la fin de cette section, on présentera une base théorique sur les modèles utilisé dans le domaine des sciences du sol, en particulier les modèles corrélatifs basés sur les réseaux de neurones artificiels (ANN) qu'on a utilisés dans ce travail pour modéliser nos résultats expérimentaux.

Cette partie est divisée en trois chapitres.

Le premier chapitre (1.1) présente la dynamique des éléments traces dans le sol.

Le deuxième chapitre (1.2) présente des informations sur les sites contaminé en éléments traces et les différents techniques de remédiation des sol contaminés.

Le troisième chapitre (1.3) explique la modélisation mathématique dans le domaine des sciences du sol.

Chapitre 1.1.

Dynamique des éléments traces dans le sol

I. Les éléments traces dans les sols

I.1. Définition des « éléments traces»

Les éléments traces métalliques correspondent aux 68 éléments minéraux de la croûte terrestre présentant une concentration inférieure à 0,1% et une densité supérieure à 5g/cm^3 (Alloway, 1995 ; Baize, 1997). Ces éléments sont distribués dans tous les compartiments de l'environnement (e.g. le sol, les plantes et les animaux). Leurs concentrations dans les tissus vivants sont normalement très faibles. Ils peuvent être séparés en deux catégories. La première catégorie comprend les éléments à l'état de trace essentiels à la croissance, au développement voire à la reproduction des organismes vivants aussi bien microscopiques que macroscopiques (Buffle, 1988; Tessier et Turner, 1995).

La deuxième catégorie comprend les éléments métalliques toxiques, non nécessaires à la croissance des organismes vivants, tels que le cadmium, le plomb et le mercure. L'ensemble de ces éléments peut être d'origine naturelle mais les activités anthropiques peuvent augmenter leurs concentrations dans les écosystèmes terrestres. Cette contamination du sol n'est pas sans effet sur ces écosystèmes. En effet elle est à l'origine d'un stress pour les animaux et les végétaux et entraine la diminution de la biodiversité et de la couverture végétale et entraine également un manque de nutriments et d'eau (Freitas et al., 2004 ; Mench et al., 2004 ; Zvereva & Kozlov, 2004). Le devenir des éléments traces dans les sols dépend de leur mobilité et de leurs réactions avec les composants organiques et minéraux du sol. La quantification et la spéciation de ces éléments traces sont donc des données indispensables à l'estimation de leur impact dans un environnement donné.

I.2. Origine et source des éléments traces dans le sol

Il y a une grande dispersion des éléments traces dans la plupart des sols du monde, les principales sources de ces éléments accumulés dans le sol étant:

- **L'origine naturelle**: les éléments traces résultent tout d'abord d'un héritage direct des matériaux parentaux comme les roches ignées ou métamorphiques et les roches sédimentaires par l'altération des minéraux primaires et secondaires comme les argiles, oxydes et carbonates (Alloway, 1995; He et al., 2005; Baize, 1997; Abou Auda *et al.* 2002 ; Wenzel *et al.* 2003 ; Baize and teracé, 2002) (Fig. 2).

- **L'origine anthropique**: l'homme est probablement l'être vivant qui modifie le plus la composition des sols en éléments traces (Bourrelier et Berthelib,1998) soit à travers des activités agricoles par l'addition au sol d'engrais, d'amendements calcaires, de produits de traitement phytosanitaires (par exemple, sels de cuivre ou arséniate de plomb) et de boues de stations d'épuration; soit par la diffusion aérienne de poussières et d'aérosols provenant notamment des activités industrielles; soit par les apports massifs localisés comprenant les apports accidentels ou résultant d'activités de longue durée sans protection contre la dispersion (fuites, lessivage, …) dans l'environnement. On observe que les activité agricoles et industrielles sont les deux sources principales des éléments traces dans les sols et elles contribuent également à l'augmentation des concentrations de ces éléments à la surface des sols (Kabata-Pendias, 2001; He et al.,2005; Sun et al., 2010; Luo et al., 2011) (Fig. 2).

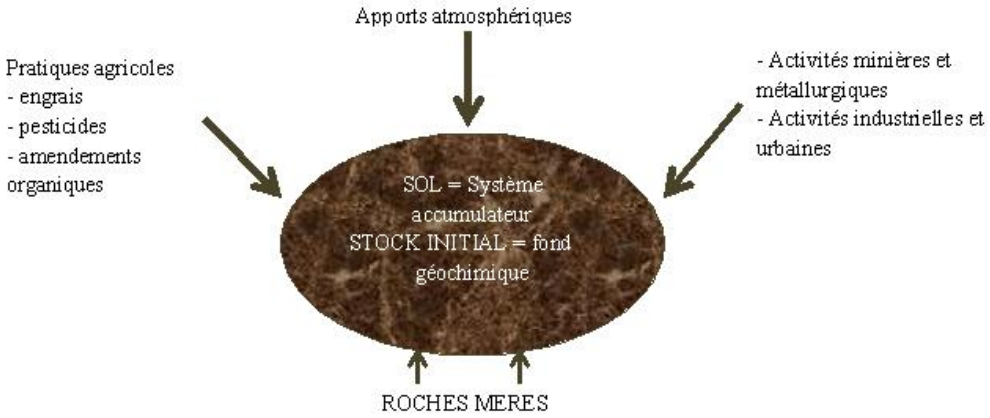


Figure 2: Origine des éléments traces dans le sol (D'après Robert et Juste, 1999)

I.3. Toxicité des éléments traces

Dans la nature, les éléments traces deviennent toxiques quand ils dépassent les limites d'adsorption spécifique pour chaque espèce vivante. Plusieurs modèles ont été proposés pour la classification de la toxicité des métaux vis-à-vis des organismes vivants. Les premiers modèles géochimiques proposés pour classifier la toxicité des métaux incluent celui de Forstner et Wittimann (1981). Ce modèle classe les éléments traces en trois catégories:

1) Les éléments **non critiques** comprenant Na, K, Mg, Ca, Fe, Li, Rb, Sr et Al

2) Les éléments **toxiques mais très insolubles** ou alors très rares comme Ti, Hf, Zr, W, Nb, Ta, Re, Ga, La, Os, Rh, Ir, Ru et Ba)

3) Les éléments **très toxiques et relativement répandus** à savoir Be, Co, Ni, Cu, Zn, Sn, As, Se, Te, Pd, Ag, Cd, Pt, Hg, Ti, Pb, Sb et Bi. De même Morgan estime que les métaux et métalloïdes anthropiques dangereux sont le Cr, Cu, Zn, Ag, Cd, Sb, Hg, Pb (Morgan, 1990).

Les effets toxiques des éléments traces sur les organismes vivants dépendent de ***la forme chimique*** des éléments traces dans le sol. Par exemple l'arsenic pentavalent sous forme d'arséniate est considéré comme plus fréquent et moins toxique que l'arsenic trivalent (Hingston et al., 2001). Ils dépendent également de ***la concentration des métaux*** dans le sol. Par exemple la mobilité, la disponibilité et par conséquent la toxicité des éléments traces dans un sol contaminé sont plus élevée qu'un sol normal. Les éléments traces font partie des contaminants les plus persistants dans l'environnement, parce que ils ne se dégradent pas contrairement aux contaminants organiques. En effet ils s'accumulent dans la chaîne alimentaire, ce qui provoque des perturbations éventuelles et des risques pour la santé humaine et l'environnement (Ruby et al., 1999 ; Kelley et al., 2002). La protection des écosystèmes terrestres par rapport à l'exposition aux éléments traces potentiellement toxiques passe premièrement par l'estimation des quantités de ces éléments dans le milieu, leur flux à travers le milieu et l'identification de leurs formes physico-chimiques pour les études éco-toxicologiques.

I.4. Spéciation, mobilité et biodisponibilité des éléments traces

I.4.1. La spéciation

Dans un milieu naturel, la spéciation chimique d'un élément est définie comme l'ensemble des formes ou des espèces chimiques de cet élément dans ce milieu. Par exemple la spéciation de ces éléments en phase dissoute est contrôlée par un certain nombre de ligands, inorganiques ou organiques, qui vont former des complexes plus ou moins stables avec ces éléments. La spéciation des éléments traces dans les sols est une des données fondamentales qui contrôlent leur migration, leur biodisponibilité et donc leur toxicité (Li et Thornton, 2001). Elle permet de préciser l'espèce, c'est-à-dire la forme ou la nature des liaisons chimiques contractées avec ses proches voisins.

A l'heure actuelle, peu de méthodes de détermination de la spéciation font l'objet d'un consensus au sein de la communauté scientifique, malgré la nécessité d'une telle approche pour la compréhension de la mobilité et de la biodisponibilité des éléments traces dans les sols. On peut distinguer deux grands types de méthodes pour déterminer la spéciation des éléments traces dans les sols:

- les méthodes physiques (ou directes)
- les méthodes chimiques (ou indirectes).

Dans le cas des méthodes **physiques,** la spéciation d'un élément considéré est abordée directement à partir de moyens analytiques non destructifs au sens où la structure ou la matrice contenant l'élément n'est pas modifiée. Parmi ces moyens analytiques figurent la diffraction de rayons X ou la microscopie analytique comme les microscopies électroniques à balayage (MEB), à transmission (MET) et la microsonde électronique qui permettent l'analyse multi-élémentaire de quelques μm^{-3} de constituants du sol (Bourrelier et Berthelin. 1998).

Pour les méthodes **chimiques**, la spéciation peut être évaluée à partir d'extractions réalisées en présence de réactifs sélectionnés ; elle sera alors définies de façon fonctionnelle (extraction simple) (Menzies et al., 2007) ou opérationnelle (extraction séquentielle) (Ure et al., 1993 ; Kersten et Förstner, 1995).

I.4.2. Mobilité et disponibilité des éléments traces dans le sol

La mobilité des éléments traces peut être définie par leur aptitude à être transférés entre les différents compartiments du sol, la phase liquide étant le compartiment ultime qui est représentée par la solution du sol. Cette dernière constitue le réservoir principal d'alimentation pour les plantes et tous les êtres vivants du sol (Kabata-Pendias and Pendias, 2001 ; McLaughlin et al., 2000). La **biodisponibilité** désigne la capacité d'un élément présent dans le sol à être absorbée par un être vivant. Lorsqu'il s'agit des plantes, on parle de phytodisponibilité (Baize et Tercé, 2002).

La mobilité des éléments traces dans les sols met en jeu des mécanismes biogéochimiques de mobilisation et des mécanismes de transport. La mobilisation et l'immobilisation recouvrent l'ensemble des phénomènes par les quelles les éléments traces sont extraits des compartiments (organiques ou minéraux) du sol où ils se trouvent.

La mobilité et la disponibilité des éléments traces sont contrôlées par les formes de leurs associations chimiques et physiques dans les sols, ces associations étant: (i) des ions simples ou complexes dans la solution du sol; (ii) des ions échangeables, (iii) des substances organiques; (iv) l'occlusion ou la co-précipitation avec des oxydes, des carbonates et des phosphates ou d'autres minéraux secondaires, et (v) des atomes dans les réseaux cristallins des minéraux primaires (Bates, 1982). Par conséquent, pour l'évaluation des risques environnementaux des éléments traces, il faut mesurer les quantités de chaque forme d'association des éléments traces, en plus de la quantité totale des éléments traces dans les sols. En effet, toutes les formes disponibles ne présentent pas forcement des risques environnementaux. Ainsi, les formes très stables contenues dans la fraction résiduelle sont peu susceptibles d'être libérées, tandis que les fractions solubles, échangeables et les espèces chélatées sont très mobiles et donc plus disponibles pour les plantes et la chaîne alimentaire (Kabata-Pendias, 1993).

II. Facteurs contrôlant la mobilité/immobilité des éléments traces dans le sol

La mobilité des éléments traces jouent un rôle majeur dans les transferts des éléments traces dans les sols; cette mobilité est contrôlée par plusieurs facteurs tels que les propriétés des sols (pH, capacité d'échange cationique (CEC), la teneur en matière organique du sol (SOM), *etc*…) et les espèces végétales (Fageria *et al.*. 2002; Hough *et al.*. 2003; Tazisong *et al.*, 2004).

Les facteurs les plus influents sur la disponibilité et la mobilité des éléments traces dans les sols sont détaillés ci-dessous:

II.1. Le pH

Le pH est le paramètre le plus important dans le sol. Il influence directement ou indirectement sur les processus de sorption/désorption, précipitation/dissolution, mobilité/immobilité et la formation de complexes et l'oxydoréduction (McLean et Bledsoe, 1992). Ce paramètre constitue le facteur-clé affectant la mobilité des ions

métalliques dans le sol (McLaughlin *et al*., 2000). D'une façon générale, l'augmentation du pH favorise la déprotonation des complexes aqueux et des groupements fonctionnels de surface des phases solides. Du fait de la diminution des quantités de protons, la compétition entre protons et cations métalliques est plus faible et donc les répulsions diminuent, ce qui accélère alors la formation de nouvelles phases. Ainsi, la solubilité des cations métalliques diminue lorsque le pH augmente. Dans le cas des anions comme (Cr(VI), As, Se), la diminution du pH favorise au contraire la libération des ions OH^- entraînant alors une diminution de la compétition entre anions et ions OH^-, ce qui accélère alors la formation de nouvelles phases.

II.2. Le potentiel redox

Le potentiel redox (Eh) est le facteur qui permet de caractériser les échanges d'électrons entre les espèces chimiques. Un potentiel redox élevé favorise la formation de formes oxydée, alors que les faibles valeurs du potentiel rédox favorisent l'apparition d'espèces réduites. Les valeurs les plus courantes du potentiel redox dans les sols naturels sont comprises entre 200 et 400 mV, ce domaine de potentiel recouvrant des degrés d'oxydation très variés de nombreuses espèces en solution, influant ainsi directement ou indirectement sur la mobilité des métaux dans ces environnements (Deneux-Mustin *et al*., 2003).

II.3. L'activité biologique

Il est difficile de comprendre les effets globaux des activités biologiques sur la mobilité/immobilité des éléments traces dans les sols car le sol est un milieu très complexe. Ainsi dans ce milieu il y a de nombreuses actions et interactions qui se produisent et à tous les niveaux des écosystèmes. Dans le sol on peut trouver les végétaux supérieurs et une forte présence de micro-organismes tels que les bactéries, les champignons et les algues. Ces nombreuses populations interviennent directement ou indirectement dans la transformation des composés minéraux et organiques et la spéciation des éléments traces par plusieurs processus comme la solubilisation, l'insolubilisation, l'altération, la volatilisation, la néoformation. Par conséquent ces processus peuvent modifier l'état des divers éléments présents dans les sols (solubilité, état rédox…) (Schlieker *et al*., 2001).

II.4. La température

La température du sol dépend en premier lieu de la météorologie et donc du climat. La température a un impact direct et indirect sur la mobilité des éléments métalliques. Un effet direct en déplaçant les équilibres des réactions de dissolution-précipitation et co-précipitation, et un effet indirect, en modifiant la teneur en eau du sol, le pH ou l'Eh (Remon, 2006).

III. Mécanismes d'interactions des éléments traces avec les constituants du sol

Le transfert des éléments traces de la phase solide vers la phase liquide et inversement est le processus le plus important qui contrôle la mobilité des éléments traces dans les sols. Plusieurs mécanismes physico-chimiques interviennent au cours de ces transferts comme l'échange ionique (adsorption non spécifique),

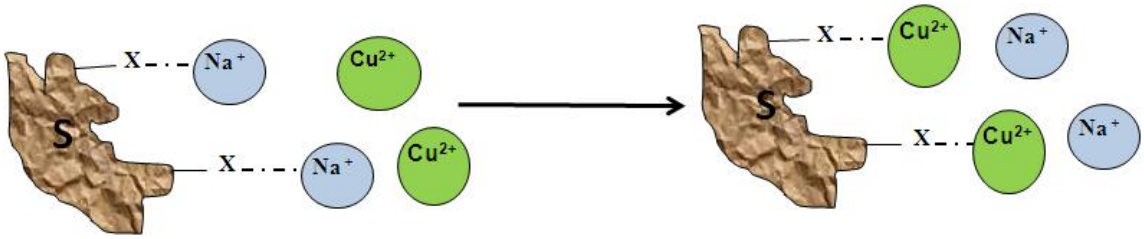
l'adsorption spécifique sur les phases minérales, la complexation avec la matière organique du sol (SOM), la précipitation et la co-précipitation (Fig. 3).

Figure 3: Différentes formes et mécanismes de fixation des éléments traces métalliques dans le sol (d'après Singh et Steinness, 1994).

III.1. Adsorption non spécifique (physisorption)

La surface des particules du sol est généralement chargée négativement. Les cations en solution sont attirés vers les charges négatives de la surface de ces particules par des forces électrostatiques de type Van der Waals ou coulombiennes. Les cations adsorbés forment par sa sphère d'hydratation avec la phase solide du sol, un **complexe de sphère externe (**Fig. 4**)**. Les liaisons entre la phase solide et les cations du sol sont des liaisons électrostatiques réversibles et soumises à la compétition avec les ions de la solution du sol pour les sites de sorption (Srivastava *et al.* 2005). Par conséquent, les éléments métalliques adsorbés sont facilement mobilisables en cas de changements physicochimiques du milieu.

Différentes particules du sol peuvent participer à l'adsorption non spécifique et former des complexes de sphère externe comme les particules argileuses (Majone *et al*., 1996), les oxydes (Martinez et McBride., 1998) et les substances humiques (Andreux, 1997 ; Hatira *et al.*, 1990).



S: surface, - liaison forte irréversible et liaison électrostatique réversible. Augmente avec le pH

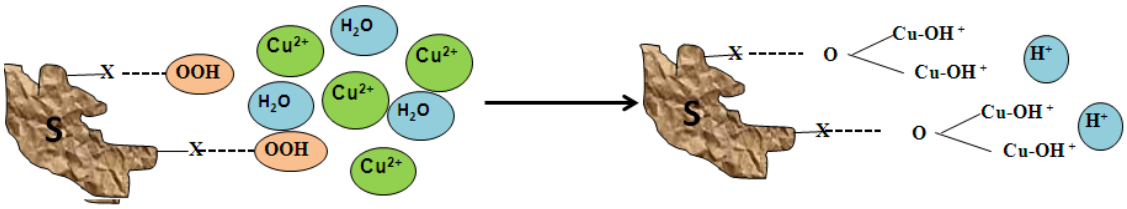
Figure 4: Formation d'un complexe de sphère externe (d'après Bes, 2008)

III.2. Adsorption spécifique (chimisorption)

Des complexes entre les cations métalliques et le substrat se forment directement après qu'il ait perdu une ou plusieurs molécules d'eau de sa sphère d'hydratation. Cela aboutit à la formation de **complexes de sphère interne** (Fig. 5). Ces complexes se forment par des liaisons ioniques, covalentes ou une combinaison des deux, plus stables que la liaison électrostatique de sphère externe. Par conséquence, les éléments métalliques adsorbés sur la phase solide sont difficilement remobilisables en cas de changements physicochimiques du milieu. Comme ce type de liaison covalente dépend de la configuration des électrons, des groupes de surface et de l'ion complexé, il est approprié de considérer la complexation de sphère interne comme une adsorption spécifique (Sposito, 1989) ou **chimisorption** (Yong *et al.*, 1992).

L'adsorption spécifique se déroule en plusieurs étapes : au début il y a l'adsorption de surface puis diffusion et finalement l'adsorption interne. L'adsorption spécifique par complexation de sphère interne fixe plus fortement les éléments traces métalliques sur les surfaces solides que la complexation de sphère externe. Le principal paramètre qui peut influencer la stabilité de ces liaisons est le pH du sol.

Cette réaction peut se produire sur tous les solides qui possèdent des groupements –OOH à sa surface tels que les groupements carboxyles des acides humiques, les polysaccharides (e.g. pectines) et polyphénols de la matière organique ou les oxydes, hydroxydes d'Al, de Fe et de Mn (Kumpiene *et al.*, 2008).



S : surface, - liaison forte et irréversible. Augmente avec le pH

Figure 5: Formation d'un complexe de sphère interne (d'après Bes, 2008)

III.3. Précipitation et co-précipitation

Les mécanismes de précipitation et de co-précipitation font partie des principaux mécanismes de rétention des éléments traces métalliques dans les sols (Sposito, 1989). Le phénomène de précipitation peut se produire soit à la surface des phases solides du sol soit dans la solution du sol. Ce phénomène traduit sur les phases solides soit par un accroissement de la surface du solide, soit la formation d'un nouveau solide à l'interface solide/liquide selon un arrangement tridimensionnel. La précipitation est contrôlée à la fois par le pH de la solution du sol et par la concentration des éléments en solution.

La co-précipitation est définie comme la précipitation simultanée d'un agent chimique conjointement avec d'autres éléments (Alloway, 1995a). Normalement cette substitution est favorisée si la taille et la valence de

l'élément substituant sont comparables à l'élément substitué.

La précipitation et la co-précipitation sont des phénomènes réversibles, pouvant intervenir à la fois dans les processus de rétention ou de désorption des éléments traces métalliques. Les principaux facteurs contrôlant la précipitation et la co-précipitation des éléments métalliques dans les sols sont : le pH du sol, celui de la solution de sol, la concentration en soluté ainsi que la température et le potentiel d'oxydo-réduction. Les éléments traces métalliques peuvent précipiter sous l'effet de ces facteurs sous forme de carbonates, de sulfures, de phosphates ou d'hydroxydes (Fig. 6).

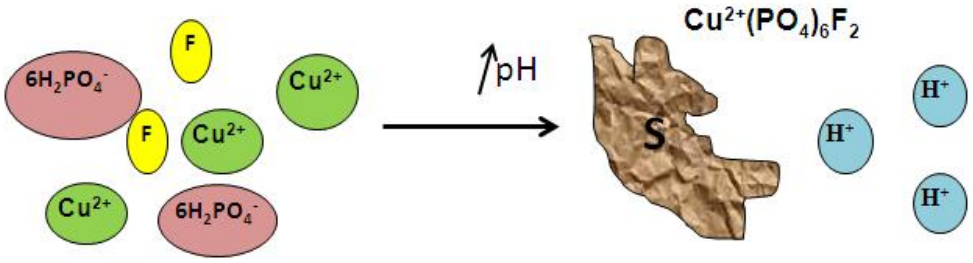


Figure 6: Précipitation du cuivre avec les phosphates (d'après Bes, 2008).

III.4. Complexation

Les ions métalliques peuvent être complexés à la matière organique du sol par association entre les cations métalliques et les groupes fonctionnels des substances humiques. Les groupes de surface de ces substances se comportent comme des ligands organiques complexants (Evans, 1989). Une réaction de complexation se produit quand un cation métallique réagit avec un anion ayant une fonction de ligand. Ces réactions sont lié aux groupements de surface basiques (-NH_2 (amine), C=O (carbonyle), -OH (alcool) et -S-OR (thioéther)) et aux groupements acides (-COOH (carboxyle), -OH (hydroxyle) et -SH (thiol). D'après Alloway (1995), les groupes carboxyles jouent un rôle prédominant dans les liaisons métal-acide humique ou acide fulvique. Bien que la liaison du métal sur la matière organique puisse être vue comme un échange d'ions entre un proton (H+) et un ion métallique sur un groupe fonctionnel acide, le fort degré de sélectivité de la matière organique pour certains métaux suggère fortement que le métal est coordonné directement (complexe de sphère interne) avec le groupe fonctionnel (McBride, 1989). De plus la complexation de sphère interne peut conduire à la complexation de sphère externe à un fort niveau de sorption.

IV. Effets des composants des sols sur la spéciation des éléments traces

La principale source de minéraux des sols est la roche mère sous-jacente. A partir de cette roche mère on distingue deux catégories des minéraux dans les sols : les minéraux primaires, hérités sans modification de la roche mère; et les minéraux secondaires, issus de la pédogénèse par des phénomènes d'altération physico-chimiques, mais aussi biologiques des minéraux primaires et des roches mères. En plus de ces deux catégories, il

existe les matières organiques héritées de l'activité des organismes vivants. L'ensemble de ces trois catégories forme la phase de rétention des métaux, en équilibre dynamique avec l'eau du sol.

VI.1. Les constituants minéraux des sols

VI.1.1. Les minéraux primaires

Les minéraux primaires sont les composants principaux des minéraux du sol hérités de la roche mère sans modification grâce aux processus de désagrégation mécanique souvent d'origine climatique comme la succession gel/dégel. D'autre part ces minéraux n'ont pas de capacité de réaction avec les éléments traces dans les sols en raison de leur résistance aux processus d'altération chimique en vigueur dans les conditions environnantes; cependant de par leur composition, leur structure et leur taille ils peuvent fournir une réserve des éléments réactifs dans les sols (Duchaufour, 1995, Lespagnol, 2003; Remon, 2006).

VI.1.2. Les minéraux secondaires

Lorsque les processus d'altération physico-chimiques et biologiques des minéraux premiers se poursuivent, des minéraux secondaires plus réactifs vont se développer directement ou indirectement lors de la pédogénèse (Duchaufour, 1995). Ces minéraux créés durant la pédogénèse sont essentiellement des argiles, des (hydr)oxydes de fer (Fe), d'aluminium (Al) et de manganèse (Mn), des carbonates, des sulfates et des phosphates. Tous ces minéraux sont des composants de très petite taille et considérés comme les principaux constituants minéralogiques des sols ayant un rôle dans la rétention des éléments traces.

VI.1.3. Les argiles

Les argiles sont les composants les plus importants des minéraux secondaires. Ils forment des phyllosilicates dont l'unité de base est un feuillet. La petite taille des argiles leur confère des propriétés colloïdales, cette petite taille et la forme en feuillets donnent à l'argile des propriétés importantes par rapport aux autres constituants des minéraux secondaires. Les propriétés principales sont les suivantes:

- Les argiles ont une structure formée d'une superposition de feuillets (phyllosilicate) qui leur confère une surface spécifique interne très variée (de 0 $m^2.g^{-1}$ pour la kaolinite à 750 $m^2.g^{-1}$ pour des vermiculites ou des smectites). Cette surface interne vient s'ajouter à une surface externe importante liée à leur très petite taille et à leur morphologie (de moins de 1 $m^2.g^{-1}$ pour les vermiculites à 50 $m^2.g^{-1}$ pour les smectites). La surface relative des argiles augmente avec la diminution du son diamètre. Sa surface est supérieure par rapport à celles de minéraux de même taille mais de forme différente. Les propriétés des argiles sont principalement contrôlées par leur surface.

- Les minéraux argileux se caractérisent par une surface électrique non neutre qui détermine leur capacité d'échange ionique. Par conséquence il existe deux types de charge sur la surface des argiles, des charges

négatives permanentes ou structurelles liées aux substitutions ioniques (Al^{3+} pour Si^{4+}, Mg^{2+} ou Fe^{2+} pour Al^{3+}) ou des charges positifs liée à l'hydrolyse de liens rompus Si-O et Al-OH le long des surfaces. Toutefois, la surface des argiles dans la majorité des cas se charge négativement ce qui donne aux argiles une grande capacité de réagir avec les métaux des sols.

VI.1.4. Les oxydes de fer, de manganèse et d'aluminium

Les oxydes, les hydroxydes et les (oxy) hydroxydes de fer, sont présents en abondance dans les sols et existent sous forme des composés amphotères ou cristallins formés d'éléments métalliques tels que le Fe, l'Al et le Mn, des atomes d'oxygènes O et groupes hydroxyles OH. Les (hydr)oxydes de fer sont les plus abondants alors que les (hydr)oxydes d'aluminium sont relativement moins fréquents, ainsi les oxydes de manganèse étant plus généralement sous forme amorphe. La réactivité des (hydr)oxydes de fer et d'aluminium provient de leur importante surface spécifique et de leur charge de surface, cette réactivité dépendant fortement du pH du sol (Subramaniam et al., 2003). En effet, pour des pH à tendance acide, les groupements OH se protonent en $–OH^{2+}$ attirant ainsi les anions, pour des pH à tendance basique, les groupements OH se déprotonent en $–O^{-}$ attirant ainsi les cations. Il existe aussi un pH où la surface est globalement neutre, ce pH est appelé pH de point de charge nulle (pH PCN) (Kabata-Pendias and Pendias, 2001; Sposito, 1989). Les processus de coprécipitation sont importants dans la fixation de certains éléments traces dans la structure même des oxydes (formation de solutions solides Fe^{3+} et Cr^{3+} par exemple). Enfin, des réactions d'oxydo-réduction induites par la surface des oxydes peuvent jouer un rôle important dans la transformation de certains métaux ou métalloïdes (réduction du Cr(VI) par Mn(II) à la surface des oxydes par exemple).

VI.1.5. Les carbonates, phosphates, sulfates, sulfures et chlorures

Dans la plupart des sols nous pouvons trouver des carbonates associées avec le Ca, Mg et Fe mais aussi associés avec beaucoup de métaux divalents présents dans les sols contaminés tels que Pb, Zn, Cu, etc. La forme la plus abondante des carbonates est la calcite (CaCO3), un composant assez soluble, qui a une influence majeure sur le pH des sols et par conséquent sur les phénomènes de sorption des métaux. En outre, les réactions de dissolution/précipitation des carbonates favorisent le piégeage des ions métalliques au sein des cristaux formés (Blanchard, 2000).

Les phosphates (groupement PO_4^{3-}) sont des minéraux peu abondants dans les sols mais susceptibles, comme les carbonates, de substitution du calcium par des éléments métalliques divalents (e.g. Pb, Zn, Cu), en particulier dans des sols à tendance basique (Kabata-Pendias and Pendias, 2001).

Les sulfates (généralement de la forme $R2+SO4^{-2}$) et les arséniates (minéraux d'arsenic As^{+5} comprenant le groupement AsO_4^{-3}), les sulfures (souvent $R+2S^{-2}$), les arséniures (minéraux d'As^{+3}) et les chlorures (principalement $R+2Cl^{-2}$) sont des espèces présentes en quantité généralement négligeable dans la plupart des sols, mais qui peuvent être abondantes en contexte industriel ou minier.

VI.2. Les constituants organiques

La matière organique du sol (MOS) se compose de différents types de composés carbonés et azotés. Une définition des MOS est donnée par Feller (1997) « La MOS est l'ensemble des constituants organiques d'un sol, morts ou vivants, d'origine végétale, animale ou microbienne, fortement transformés ou non ». A partir de cette définition ces composés se répartissent **d'un point de vue biologique** en deux fractions:

i) une fraction organique **vivante** représentent environ 15 % des matières organique des sol (MOS) et regroupent tous les microorganismes (bactéries, champignons, algues et actinomycètes), la pédofaune (protozaires, acariens, vers de terre, nématodes, insectes) et les racines des végétaux (Olah *et al.,* 1978), ces constituants vivants étant regroupés sous le terme de microflore. Son influence sur la fixation des éléments traces est importante. Elle participe largement aux modifications de certains facteurs pédologiques affectant la fixation et la mobilité des éléments traces dans le sol comme le potentiel redox ou le pH (Kabata-Pendias and Pendias, 2001).

ii) une fraction organique **non vivante** formant environ 85 % des MOS et représentant des fractions mortes (débris d'origine animale ou végétale) plus ou moins décomposées en humus. Ainsi, on retrouve les cires, les lipides, les protéines, les peptides, les acides organiques, les acides aminés ou encore les hydrocarbures aromatiques polycycliques (HAP) (Kabata-Pendias and Pendias, 2001). Ces composés s'associent entre eux pour former les substances humiques (humine, acides humiques, acides fulviques) piégeant différents éléments traces dans les sols et contrôlant les échanges ioniques et des cycles géochimiques des éléments métalliques. Ces fractions non vivantes regroupent les matières organiques particulaires (MOP) et dissoutes (MOD).

VI.2. 1. Les Matières Organiques Particulaires (MOP)

Cette fraction est également appelée débris ou Fraction Légère (LF) suivant (Gregorich *et al.,* 2006) ou matière organique non complexée (Christensen, 2001). Elle représente environ 10-40% des MOS. Elle correspond à des matériaux organiques frais, figurés, constitués de morceaux de racines, d'animaux, de feuilles etc. La proportion des MOP dans les sols est liée à plusieurs facteurs tels que le type de végétation, les pratiques agricoles, le climat, le type de sol et l'activité de la microfaune (Balesdent *et al.,* 1998; Christensen, 2001). Ces MOP peuvent être isolées des sols par fractionnement granulo-densimétrique dans une gamme de taille de particules > 50 µm selon Balesdent *et al.* (1991).

Ces MOP ont été étudiées en particulier pour leur labilité, leur temps de résidence dans les sols (Balesdent, 1996) et pour leurs teneurs en métaux dans les sols plus ou moins contaminés. Les travaux montrent que ces MOP sont plus riches en éléments traces par rapport à leur teneur dans le sol (Balabane *et al.,* 1999 ; Besnard *et al.,* 2001).

VI.2. 2. Les Matières Organiques Dissoutes (MOD)

Ces fractions organiques ont été décrites comme le composé final de la dégradation de la matière organique. Ces MOD ont a priori une composition très hétérogène, du fait de la grande diversité des sources et des différents facteurs (dégradation, coagulation, etc.). Elles correspondent également à des composés organiques stables, à noyaux aromatiques et riches en radicaux libres et constitué d'un faible pourcentage de composés organiques facilement identifiables. Elles sont composés principalement de molécules organiques de faible poids moléculaire telle que les acides aminés, protéines, lipides, sucres, etc, avec un pourcentage important de composés réfractaires difficilement identifiables composés de macromolécules complexes de haut poids moléculaire comme les substances humiques comprenant les acides humiques, acides fulviques, humines, etc…. (Harter et Naidu, 1995 ; Kalbitz *et al*., 2000 ; Strobel, 2001 ; McDonald *et al.*, 2004). Certains composés des MOD comme les acides humiques, acides fulviques sont connus pour leur affinité avec les métaux et participent donc ainsi à leur mobilisation vers les différents horizons des sols (Citeau, 2004). L'extraction des substances humiques des sols SH est réalisée par des solutions alcalines, cette extraction conduisant à des solutions contenant un grand nombre de molécules de taille et de composition très variées.

Après une simple extraction aqueuse, trois fractions de substances humiques sont classiquement mises en évidence (Mac Carthy *et al.*, 1990; Stevenson, 1982; Stevenson and Cole, 1999) (Tab. 1).

1) **Les acides humiques (AH)** représentent la fraction majeure des substances humiques, (Brunetti *et al.*, 2006); ils sont des polymères à haut poids moléculaire entre 5000 - 10000 Da, chargés négativement, de couleur noire à brun foncé, solubles en milieu basique et insolubles dans le domaine des pH acides (pH<2). Ils sont riches en carbone mais moins riches en oxygène (Dommergues et Mangenot, 1970; Flaig, 1970). Les acides humiques se caractérisent par un noyau plus important et des chaînes aliphatiques latérales plus courtes.

2) **Les acides fulviques (AF)** L'acide fulvique a un faible poids moléculaire compris entre 500 - 2500 Da. Ils possèdent un taux de carbone relativement faible. L'oxygène est abondant et présent sous forme de groupes fonctionnels responsables d'une acidité élevée (Dommergues et Mangenot, 1970; Flaig, 1970). Ces acides se caractérisent par un noyau moins important que le noyau des acides humiques et des chaînes aliphatiques latérales plus longues. Ils sont de couleur jaune, soluble dans l'eau quelle que soit la valeur de pH. Ils seraient considérés à la fois comme précurseurs et produits des acides humiques (Tate, 1987; Tissaux, 1996).

3) **L'humine,** C'est la plus grosse molécule humique du sol et représente la partie non extractible des substances humiques humifiées. Ils ressemblent beaucoup aux acides humiques mais diffèrent seulement par le fait qu'elles se trouvent en association très étroite avec les matériaux inorganiques (Allison, 1973; Swift *et al.*, 1979). Les fractions des humines sont de couleur noire, insoluble dans l'eau quelle que soit la valeur de pH. A cause de cette raison l'humine est peu connue et il n'y a pas beaucoup de publications sur ce constituant.

L'humine possède également un noyau beaucoup plus important que l'acide humique et de courtes chaînes, ce qui lui confère une grande stabilité.

Tableau 1: Caractéristiques des substances humiques (Stevenson and Cole, 1999)

	Acides fulviques	**Acides humiques**
Couleur	Jaune	brun
Degré de polymérisation	faible	élevé
Poids moléculaire (Da)	< 1000	300 000
Carbone	45%	62%
Oxygène	48%	30%
Acidité échangeable (cmol/kg)	1400	< 500
Degré de solubilité	élevé	faible

VI.2. 3. Rôle des substances humiques sur la dynamique des éléments traces dans le sol

La présence des substances humiques dans le sol influent positivement sur la qualité et la fertilité du sol en améliorant sa structure, en augmentant l'activité biologique, la disponibilité des nutriments et en complexant les métaux toxiques en réduisant leur mobilité et biodisponibilité et le risque de propagation dans les chaines trophiques et alimentaires (Harter et Naidu., 1995 ; Stevenson, 1985).

Ces substances humiques chargées négativement sont très réactives vis-à-vis des métaux. Elles peuvent en effet complexer les éléments traces métalliques grâce à leurs groupes carboxyliques et phénoliques (Schnitzer et Skinner, 1965; Piccolo et Stevenson, 1982). La formation de ces complexes augmentent la capacité d'échange cationique des sols et limitent le lessivage de certains éléments (Vaughan et Ord, 1985), permettent la détoxication en complexant les éléments traces et améliore également la biodisponibilité des éléments minéraux pour les végétaux en limitant leur précipitation (Revel *et al.,* 1999; Stevenson, 1985).

Les dynamiques des éléments traces et celles des MOS sont clairement liées : la réactivité des MOS vis à vis des éléments traces est bien connue. On sait par exemple que la présence des éléments traces dans les sols a généralement un effet toxique sur la dynamique des MOS (Kandeler *et al.,* 1996; Ross, 1994) Ainsi les substances humiques peuvent former avec les métaux différents types d'associations suivant le métal impliqué et sa concentration (Hatira *et al.,* 1990; Murray et Linder, 1983; Andreux, 1997). Les principaux groupes complexant vis à vis des métaux, classés dans l'ordre décroissant d'affinité sont les suivants (Schnitzer et Kahn, 1972; Calvet, 2003) : -O- (énol) > -NH2- (amine primaire) > -N=N- (azo) > = N aromatique >-COO- (carboxyl) > - O- (éther) >> -C-O (carbonyl).

La complexation d'un cation métallique par les substances humiques dépend non seulement de la constante de stabilité du complexe métal-ligand, mais aussi de la constante de dissociation du ligand protoné (Lamy, 2006). L'affinité des cations métalliques pour les ligands dépend de leur structure électronique ; le classement suivant représente l'ordre de stabilité des complexes avec les substances humiques (Calvet, 2003) :

$Pb^{2+} > Cu^{2+} > Ni^{2+} > Co^{2+} > Zn^{2+} > Cd^{2+} > Fe^{2+} > Mn^{2+} > Mg^{2+}$. Il y a en effet compétition, au niveau des ligands organiques, entre les ETM et les protons (Berthelin *et al.*, 1998).

Dans les sols, les éléments traces se localisent principalement dans les fractions fines les plus actives dans les sols (Ducaroir et Lamy, 1995 ; Flores-Vélez *et al.*, 1996 ; Besnard *et al.*, 2001). Ces fractions fines sont généralement riches en carbone organique, fixé à ces fractions grâce à des processus de l'adsorption physique ou chimique de molécules organiques à la surface des minéraux (Yariv et Cross, 2002). Cette association organo-minérale rend la séparation physique de la matière organique et de la matrice minérale très complexe. La meilleure approche pour pouvoir étudier le complexe organo-minérale reste cependant l'extraction chimique des substances humiques en milieu alcalin. Cette séparation nous permettra de mieux comprendre la localisation et l'affinité des métaux dans et vers les fractions du sol.

Chapitre1.2.

Les sites contaminés en éléments traces et les différentes techniques de remédiation des sols contaminés

I. Définitions

Le sol est un milieu très important dans la vie. Il joue un rôle déterminant : produire les aliments, réguler le cycle et la qualité de l'eau, accumuler du carbone et limiter l'effet de serre, recycler les matières organiques, entretenir la biodiversité, fournir des matériaux pour la construction et l'industrie, participer à la valeur esthétique des paysages (INRA, 2009).

La première définition donnée par Dokouchaev en 1883 (Gobat *et al.*, 2010) est la suivante. Par sol, on entend les horizons extérieurs des roches naturellement modifiés par l'influence mutuelle de l'eau, de l'air et des organismes vivants et morts ; c'est un corps naturel indépendant et variant. Cette définition a évolué au cours du temps et nous retiendrons celle de Ruellan et Poss (2008) qui énonce simplement que le sol provient de la décomposition et de l'altération des roches par l'action de l'eau, de l'air et des être vivants. Au cours du temps, le sol s'épaissit et se modifie; il acquiert des constituants (matières organiques, argiles, …) et des structures (couleurs, agrégats, horizons etc.) qui lui sont spécifiques.

D'après Baize et Jabiol (2011), ce qu'on appelle couramment « le sol » ou « les sols » est beaucoup mieux désigné par le terme « couvertures pédologiques ». Les couvertures pédologiques sont des objets naturels dont l'existence et l'état actuel résultent de l'évolution au cours du temps d'un matériau minéral sous l'action combinée de facteurs climatiques (température, précipitations) et de l'activité biologique (végétaux, animaux, microorganismes).

Le sol peut être transformé par l'homme ce que nous appelons ***anthroposol***. D'après le Référentiel Pédologique 2008 (RP) les anthroposols sont des sols fortement modifiés ou fabriqués par l'homme, souvent en milieu urbain mais aussi, dans des conditions particulières, en milieu rural.", "Du fait qu'ils ont été modifiés ou fabriqués durant la période historique, les anthroposols sont généralement considérés comme « jeunes » et peu évolués." Le RP a donné référence à 5 types d'anthroposols : les anthroposols transformés, les anthroposols artificiels, les anthroposols reconstitués, les anthroposols construits et les anthroposols archéologiques (Hu, 2012).

Dans la classification internationale (WRB, 2006), les *Anthroposols* sont équivalents aux *Technosols* d'après la classification internationale (WRB, 2006) des sols avec une légère différence en définition. Les

Technosols sont des sols dont les propriétés et la pédogenèse sont dominées par leur origine technique, autrement dite anthropique. Ils se caractérisent soit par de fortes quantités de matériaux artificiels (définis comme des artefacts) soit par un scellement anthropique en surface ou en profondeur (Séré, 2007). Pour qu'un sol soit un technosol, il doit contenir au moins 20 % (en volume ou en moyenne pondérale) de matériaux artificiels dans les 100 premiers cm, quel que soit son épaisseur.

Il est indispensable de bien différencier la part des éléments traces d'origine naturelle, de celle qui résulte de contaminations d'origine humaine (anthropogène). Les métaux d'origine anthropiques sont souvent assez réactifs et capables de générer des risques très supérieurs aux métaux d'origine naturelle qui sont le plus souvent sous des formes immobilisés. Différentes définitions explicitant le concept du sol contaminé et/ou pollué sont présentes dans la littérature. Il n'y a cependant pas de définition standardisée internationale (ISO) pour les distinguer. Ces deux termes sont souvent confondus et employés l'un pour l'autre. Le rapport n°42 de l'Académie des Sciences (Bourrelier et Berthelin, 1998) définit dans son glossaire ces termes de la manière suivante :

- *Contamination* : Elle qualifie un apport d'éléments en traces issus de l'extérieur du site qui a pour conséquence une élévation de leur teneur originelle. Cette expression n'indique pas des conséquences (risques) que pourrait avoir cette élévation de la teneur en éléments en traces sur le comportement des organismes vivants qui utilisent ce site.
- *Pollution* : Contamination qui a pour conséquence une perturbation du milieu ou de l'usage qui en est fait habituellement.

D'après Baize, 1997, le terme « contamination » doit être employée pour les sols lorsqu'il y a des apports anthropiques importants mais sans effet apparent pour l'environnement. Par contre, il préconise le terme « pollution » lorsque des apports liés à des activités humaines ont des effets négatifs visibles sur l'environnement.

D'autres auteurs ont mis l'accent sur d'autres aspects de ces deux termes :

- *Contamination*: Présence anormale d'une substance, de micro-organismes dans un milieu, un objet, ou un être vivant. La notion de contamination est appliquée historiquement et dans l'ordre aux micro-organismes, puis aux substances radioactives (BRGM, 2000).
- *Pollution*: Introduction, directe ou indirecte, par l'activité humaine, de substances, préparations, de chaleur ou de bruit dans l'environnement, susceptibles de contribuer ou de causer :

— un danger pour la santé de l'homme ;

— des détériorations aux ressources biologiques, aux écosystèmes ou aux biens matériels ;

— une entrave à un usage légitime de l'environnement (ISO 11074-4, 1999).

Finalement, l'existence d'anciens dépôts de déchets ou d'infiltration de substances polluantes peut générer des ***Sites pollués*** qui présentent une pollution susceptible de provoquer une nuisance ou un risque pérenne pour les personnes ou l'environnement. Ces situations sont souvent dues à d'anciennes pratiques sommaires d'élimination des déchets, mais aussi à des fuites ou à des épandages fortuits ou accidentels de produits

chimiques (ADEME, 2012). En fonction de l'intensité et l'origine de ces polluants les chercheurs ont distingué des grands types de pollution des sols qui sont :

- **Les pollutions accidentelles:** Elles proviennent généralement d'un déversement ponctuel et momentané de substances polluantes. Cette pollution engendre, en règle générale, d'abord une dégradation du milieu sur une surface limitée, si aucune intervention n'est réalisée dans un délai relativement court, la pollution peut alors migrer vers le sous-sol.

- **Les pollutions chroniques :** Survenant sur de longues durées, elles ont souvent pour origine des fuites sur des conduites ou autres réseaux enterrés, sur des cuvettes de stockage non parfaitement étanches, mais aussi des lixiviats issus de dépôts de déchets ou de produits.
- **Les pollutions diffuses :** Souvent générées par l'épandage de produits solides ou liquides (emploi d'engrais ou de pesticides en agriculture) ou les retombées atmosphériques, elles se développent sur de grandes surfaces. La dispersion puis l'accumulation de substances dangereuses sur ces sols donnent ainsi des *sites uniformément contaminés* (NF ISO 11074-1,1997).
- **Les pollutions localisées**, À l'inverse des pollutions diffuses, elles se distinguent par la présence ponctuelle dans les sols et sous-sols de substances dangereuses provenant généralement de déversements, de fuites ou de dépôt de déchets. Non confinées et en fortes concentrations, ces substances donnent naissance à des ***sites localement contaminés*** (NF ISO 11074 -1,1997) (http://www.ademe.fr/midi-pyrenees/a_6_01.html).

II. Les sites contaminés en France

Le Ministère chargé de l'Environnement recense plus de 4336 sites et sols pollués ayant accueilli une activité industrielle polluante (Fig. 7) et pour lesquels l'Etat français a entrepris une action de remédiation. Ils sont susceptibles de présenter un risque pour la santé des riverains s'il n'y a pas eu dépollution ou si celle-ci a été mal réalisée. Leur localisation est bien sûr liée au passé industriel de chaque région : sidérurgie en Lorraine, extraction d'uranium dans le Limousin, dépôts d'hydrocarbures sur les côtes du Languedoc ou à proximité de Marseille etc. Les professionnels estiment qu'il reste nombre de terrains pollués encore inconnus.

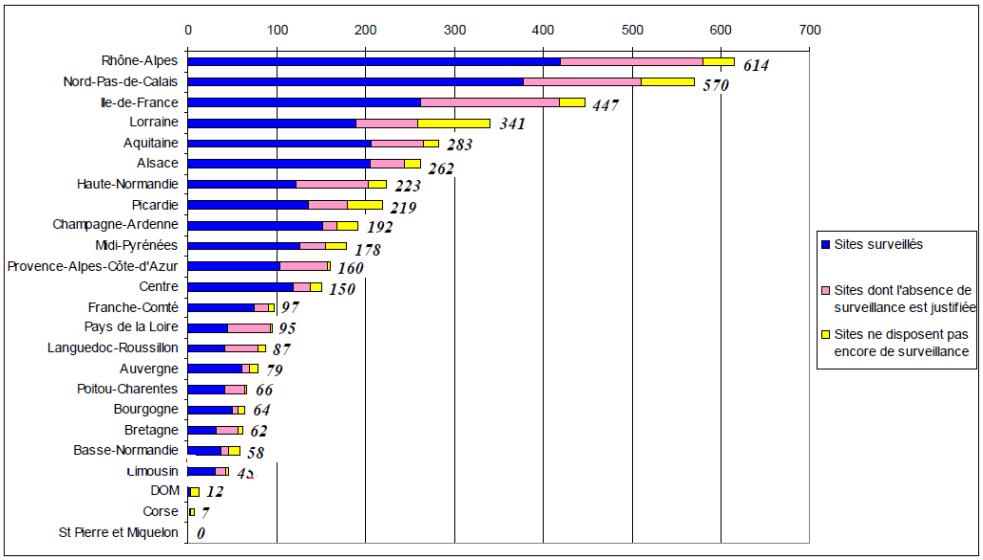


Figure 7: Nombre de sites et sols pollués par région au 23/04/2012. (Les chiffres indiquent le nombre total de sites et sols pollués, source : Basol)

En termes d'occurrence, les principaux polluants constatés sont présentés dans la figure 8. On remarque que, comme d'autres pays d'Europe, la plus grosse source de pollution en France est encore les éléments traces, même avec une influence beaucoup plus grande que la moyenne d'Europe (51,2 % en France contre la moyenne d'Europe de 37,3 %).

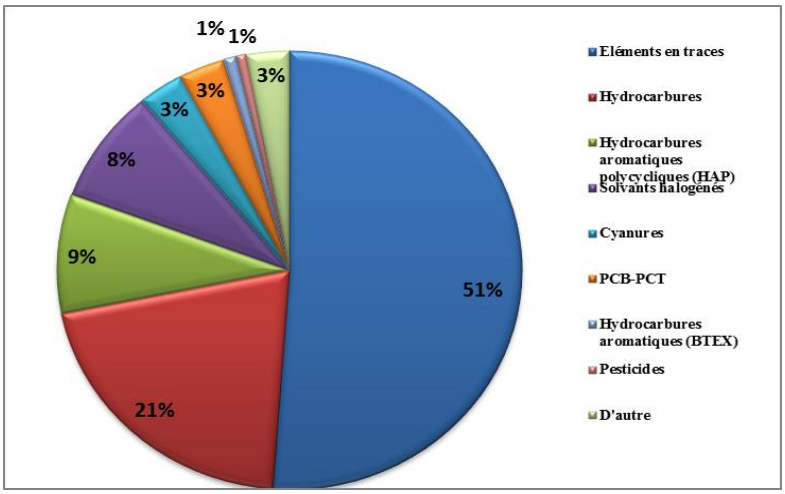


Figure 8: Les principaux polluants détectés dans les sols et les eaux souterraines en France (Chiffre en %, source : Basol, consulté le 23/04/2012)

III. Techniques de rémédiation

Les sols contaminés peuvent entraîner de graves conséquences pour l'environnement, telles que la perte des écosystèmes et de la productivité agricole, la détérioration de la chaîne alimentaire, la contamination des

ressources en l'eau et des dommages économiques dans plusieurs parties du monde. Cependant pour diminuer l'effet négatif des contaminants, il est très important d'éliminer les concentrations excessives de ces polluants (éléments traces dans notre cas) du sol. Actuellement, il existe plusieurs technologies qui peuvent être utilisées pour immobilier ou éliminer les éléments traces des sols contaminés et des déchets miniers, ces techniques variant selon les pollutions rencontrées. Elles peuvent être classées en 4 grandes catégories:

- les procédés physico-chimiques,
- les procédés thermiques,
- les procédés biologiques,
- le confinement.

Les principes d'application des différentes méthodes se regroupent en 3 modes de fonctionnement :

- traitement "in situ", cette méthode permet de traiter les déchets et les matières polluées sans qu'il ne soit nécessaire d'effectuer une excavation,
- traitement hors site, les déchets et les matières polluées sont emmenés hors du site, transportés jusqu'à un centre spécialisé pour y être traités,
- traitement sur site ou "on site", dans ce cas une excavation est effectuée, mais le traitement se fait sur place.

III.3. Remédiation des sols contaminés en éléments traces In situ par des techniques de stabilisation

Pour améliorer la qualité des sols contaminés, des techniques de stabilisation des éléments traces dans les sols contaminés sont en cours d'élaboration. Ces techniques peuvent être utilisées in situ et ex situ pour remédier les zones dévastées et industrielles, restaurer les propriétés physiques, chimiques et les propriétés biologiques du sol et de réduire la mobilité et la biodisponibilité des contaminants par divers amendements chimiques et minéralogiques. Cette approche est l'une des méthodes utilisées pour réduire les effets négatifs des contaminants tels que As, Cr, Cu, Pb, Cd et Zn sur les récepteurs de l'environnement tels que les microorganismes, les plantes, les animaux, les plans d'eau et les humains (Lombi *et al.*, 2002).

Les techniques d'assainissement in situ sont considérées comme une approche simple et efficace pour remédier les sols contaminés, lorsque ces sols sont difficiles ou coûteux à être enlevés et traités ex situ. L'objectif principal de cette technique est de ne pas réduire la teneur totale de contaminants mais de contribuer à diminuer la fraction qui potentiellement mobiles ou biodisponible des éléments ou des composés toxiques (Mench *et al.*, 2000; Oste *et al.*, 2002; Bolan et Duraisamy, 2003; Adriano *et al.*, 2004; Pérez de Mora *et al.*, 2005; Raicevic *et al.*, 2005; Kumpiene *et al.*, 2006; Kumpiene *et al.*, 2008). L'application d'amendement(s) dans un sol contaminé induits des changements dans l'état physico-chimique et / ou chimiques des contaminants (Mench *et al.*, 2000, 2003, 2006; Williford *et al.*, 2001; Brown *et al.*, 2005; Kumpiene *et al.*, 2006) ce qui

conduit à la formation d'espèces insolubles, absorbées ou d' espèces chimiques qui ont la capacité à piéger les éléments traces dans les sols, et finalement réduire ainsi les effets de ces métaux sur les récepteurs de l'environnement (Boisson *et al.*, 1999; Geebelen *et al.*, 2003; Ruttens *et al.*, 2006). Cette technique a plusieurs avantages et inconvénients, mais le principal **avantage** est le simple mélange de plusieurs amendements de sol afin de réduire la mobilité et la biodisponibilité des éléments traces dans les sols contaminés. **L'inconvénient** de cette approche est que le produit final de l'assainissement, qui contient les contaminants des sols immobilisé, qui existe sous forme inactive, reste dans le sol (Raicevic *et al.*, 2005). Pour fixer les contaminants dans les sols, deux techniques ont été proposées : 1) stabilisation des contaminants en utilisant des amendements ***minéraux*** ; 2) stabilisation des contaminants en utilisant des amendements ***organiques*.**

III.1.1.Remédiation des sols contaminés par des amendements minéraux

De nombreux amendements peu coûteux et efficaces ont été testés et proposés pour stabiliser in situ les éléments traces, comme des produits agricoles, des matériaux alcalins (calcite, chaux, dolomite et des scories)(Geebelen et al., 2003), les oxydes minéraux de phosphate (acide phosphorique, phosphates, apatites de synthèse) (Basta et McGowen, 2004), les carbonates, les sulfates et ainsi que divers produits industriels, tels que les minéraux alumino-silicatés (zéolithes et d'argile) (Oste et al. 2002), de boues rouges (Lombi *et al.*, 2002; Gray *et al.*, 2006) des nanoparticules comme (oxydes du fer et fer zéro valent) (Nagim, 2009). Ces amendements sont capables de réduire la mobilité des éléments traces dans le sol, leur absorption par les plantes et donc leur phytotoxicité.

III.1.2. Rémediation des sols contaminés par des amendements organiques

La matière organique est considérée comme l'un des meilleurs constituants pour retenir les éléments traces dans les sols. Normalement le sol a la capacité de réduire la mobilité et la biodisponibilité des éléments traces par plusieurs processus naturels comme la sorption, la précipitation et la complexation, ces processus étant très lents. Nous pouvons cependant accélérer ces processus grâce à l'ajout de matières organiques sur les sols contaminés ; ces matières organiques sont faciles à appliquer et peuvent couvrir des grandes surfaces de sols. De plus cette approche est mieux acceptée de la part du public plus que les autres stratégies de remédiation. Les matières organiques jouent un rôle majeur dans la remédiation et la restauration des sites contaminés par des éléments traces tel que:

1) Les matières organiques peuvent piéger directement les éléments traces par leurs surfaces qui sont chargées négativement, ce qui rend le métal moins disponible pour les êtres vivants (Adriano, 2001). Bolan and Duraisamy, 2003 ont constaté que les matières organiques peuvent augmenter la capacité d'échange des anions et cations et réagir avec les métaux dans le sol comme le Cu.

2) Elles peuvent augmenter la porosité du sol, ce qui contribue à la ventilation les sols ce qui augmente le processus de réduction et facilite la précipitation des métaux sous forme d'oxyhydroxides.

3) Elles peuvent augmenter également l'alcalinité du sol, ce qui favorise la précipitation des métaux dans les sols contaminés

4) L'ajout d'amendements organiques améliore la qualité des sols par le développement de la construction du sol et l'activité biologique des microorganismes des sols, ce qui augmente la stabilisation des cycles écologiques naturels.

5) L'apport de calcium aide à réduire la toxicité de certains ions métalliques grâce à la réduction de la capacité d'absorber ces éléments par les cellules des êtres vivants.

III.4. Remédiation les sols contaminés par les plantes (phytoremediation)

La phytoremédiation a d'abord été définie comme l'utilisation des plantes pour réduire, dégrader ou immobiliser des contaminants organiques du sol, de l'eau ou de l'air provenant d'activités humaines. Cette technique permet également de traiter des pollutions inorganiques (éléments traces, radionucléides) (Salt et al., 1995; Chaney et al., 1997; Raskin et al., 1997; Alkorta et al., 2004; Krämer, 2005). Il faut noter que des contaminants organiques peuvent être dégradés tandis que les polluants inorganiques peuvent seulement être dilués, concentrés ou déplacés mais pas éliminés car ils ne sont pas biodégradables. Le principe de cette technique repose essentiellement sur les interactions entre les plantes, le sol et les micro-organismes du sol.

Actuellement, les stratégies de phytoremédiation sont largement acceptées notamment toutes les méthodes basées sur l'utilisation de végétaux supérieurs dans un sol contaminé en but de la décontamination ces sols. Malgré leur développement récent, ces méthodes sont variées et des résultats encourageants ont d'ore et déjà été obtenus pour des problématiques diverses. Plus précisément, les quelques études réalisées à ce jour permettent d'envisager quatre grand types de stratégies de phytoremédiation particulièrement prometteuses (Fig. 9):

(1) La phytostabilisation : Utilisation des plantes tolérantes ou hypertolérantes aux contaminants présents dans le sol pour réduire leurs biodisponibilités par les écoulements (latéraux ou en profondeur) ou immobiliser les composés polluants en les liant chimiquement par précipitation, stabilisation, absorption ou piégeage de ceux-ci par la plante. Cette technique permet d'éviter la migration des contaminants par le vent, l'eau, l'érosion ou la dispersion dans les sols (Cunningham et al., 1995).

(2) La phytoextraction : C'est l'utilisation de plantes pour extraire des contaminanbts du sol. Ces plantes transportent et concentrent les contaminants du sol dans les parties récoltables de la plante. Suivant l'espèce de la plante et la nature du métal, les teneurs les plus élevées sont observées, soit au niveau des parties souterraines, soit au niveau des parties aériennes (McGrath, 1998, Remon 2006).

(3) La phytostimulation : Les plantes sécrètent des exsudats racinaires qui peuvent être utilisés par les communautés microbiennes et promouvoir leur développement et leurs activités. Cette stimulation microbienne

dans la rhizosphère modifie la bioaccumulation, l'oxydation/réduction biologique et éventuellement la biométhylation des éléments traces au niveau des racines (rhizosphère) notamment via l'augmentation de l'infiltration des eaux et à l'aération lors du développement du système racinaire (McGrath, 1998).

(3) La Phytostimulation : Les plantes sécrètent des exsudats racinaires qui peuvent être utilisés par les communautés microbiennes et promouvoir leur développement et leurs activités. Cette stimulation microbienne dans la rhizosphère modifie la bioaccumulation, l'oxydation/réduction biologique et éventuellement la biométhylation des éléments traces au niveau des racines (rhizosphère) notamment via l'augmentation de l'infiltration des eaux et à l'aération lors du développement du système racinaire (McGrath, 1998).

(4) La phytovolatilisation : Utilisation de plantes qui absorbent des contaminants organiques et autres produits toxiques, transformant ceux-ci en éléments volatiles peu ou pas toxiques et les relâchant dans l'atmosphère via leurs feuilles (McGrath, 1998). Cette approche est encore au stade expérimental, elle fait actuellement l'objet de recherches très actives. Les résultats récents (Bizily et al. 1999) dans ce domaine sont particulièrement encourageants (Bes, 2008).

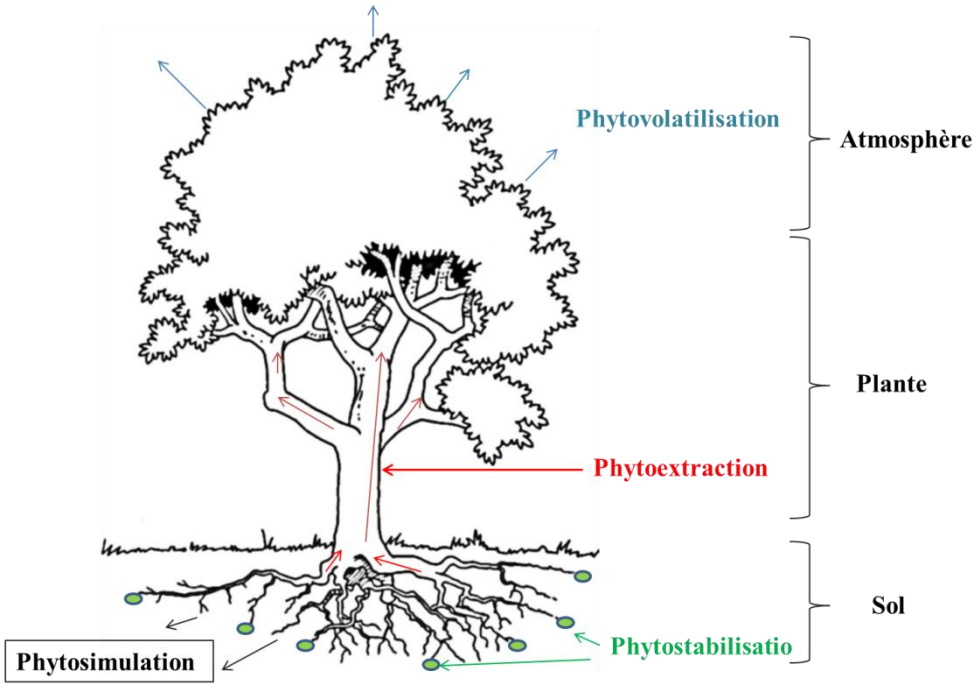


Figure 9: Différentes techniques de phytoremédiation

III.2.1. La phytoextraction

Parmi les différentes méthodes de phytoremédiation, la technique de la phytoextraction est la plus utilisée. Elle est appliquée particulièrement pour la dépollution des éléments traces. La phytoextraction est une technique attractive, car elle est peu agressive pour l'environnement et relativement peu coûteuse par rapport aux méthodes traditionnelles qui perturbent les écosystèmes. La phytoextraction est une technique qui utilise des

plantes capables de prélever les éléments traces. Ces contaminants vont s'accumuler dans les parties aériennes de la plante via la lignification et dans les vacuoles de cellules des feuilles. Apres l'accumulation des contaminants dans les plantes qui seront ensuite récoltées, les tissus végétaux ayant concentré les éléments traces seront traités par séchage, incinération ou compost (Mench et al., 2009; Vangronsveld et al., 2009; Zhao and McGrath, 2009)

La plante idéale pour la phytoextraction doit être capable d'accumuler et de tolérer de fortes teneurs en métaux dans ses parties récoltables, tout en ayant une croissance rapide et une forte biomasse (Chaney et al., 1997). Plusieurs types de plantes accumulatrices sont utilisées pour nettoyer les sols contaminés en éléments traces. Les plus efficaces sont les plantes dites **hyperaccumulatrices** ; ces plantes sont capables d'accumuler plus de 10 000 $mg.kg^{-1}$ de Mn ou Zn, plus de 1000 $mg.kg^{-1}$ de Cu, Co, Ni, Pb, Cr, ou plus de 50 $mg.kg^{-1}$ de Cd dans leurs feuilles (Van Nevel et al. 2007; Munn et al. 2008; Dickinson et al. 2009; Kupper et al. 2009; Vangronsveld et al. 2009). Cependant, ces plantes sont généralement de petite taille et ont une croissance lente. Ils peuvent accumuler les métaux essentiels tels que Fe, Mn, Zn, Cu, Mg, Mo, nécessaires à leur bon développement, mais sont également capables d'accumuler des métaux toxiques tels que Cd, Cr, Pb, Co, Ag, Se, Hg, qui, eux, ne sont pas nécessaires au niveau physiologique.

Cette technique peut utiliser des plantes ayant un intérêt économique comme des arbres (chauffage), les tournesols (essences). Il est cependant souhaitable de recycler les biomasses végétales pour récupérer les contaminants qu'elles contiennent avant de les utiliser (Pilon-Smits, 2005). L'utilisation des plantes peut en effet créer une nouvelle source d'exposition avec l'entrée des contaminants dans la chaine alimentaire et un retour au sol avec la chute des feuilles et la formation des litières (Stoltz et Greger 2002 ; Bes, 2008). Cette technique peut être améliorée en ajoutant des amendements au sol (phytoextraction aidée) pour rendre les contaminants plus solubles et donc plus disponibles pour les plantes; ce sont des agents chélateurs ou acidifiants (Prasad et Freitas, 2003; Peng et al., 2005).

Le succès de la phytoextraction comme technologie de décontaminationdépend de plusieurs facteurs, y compris l'étendue de la contamination des sols, la disponibilité des métaux pour l'absorption dans les racines (biodisponibilité), et la capacité d'absorption et accumulation les métaux dans les pousses (Ernst, 1996). En fin de compte, le potentiel de phytoextraction dépend de l'interaction entre le sol, le métal et les plantes contrôlée normalement par les conditions climatiques. Il existe deux stratégies de phytoextraction, la phytoextraction assistée (induite) et la phytoextraction continue (Salt et al., 1998 ; Cooper et al., 1999).

III.2.1.1. La phytoextraction continue

Cette stratégie de phytoextraction dépend principalement du type de plante utilisée. Pour pratiquer la phytoextraction continue les plantes utilisées doivent se caractériser par leurs capacités d'extraction, d'accumulation, de translocation (transfert du polluant des racines vers les feuilles) des quantités importantes de polluants dans leurs organes, ainsi que sa capacité à résister à de fortes teneurs en métaux. Ces teneurs très

supérieures aux niveaux habituellement rencontrés ont donné leur nom aux plantes *hyperaccumulatrices*, et la notion d'*hyperaccumulation (*Mijovilovich et al., 2009).

Brooks et al. (1977) ont utilisé le terme « hyperaccumulateur = hyperaccumulatrice » la première fois pour décrire des plantes avec une teneur en nickel supérieure à 0,1% de leur matière sèche, ce qui correspond à une valeur nettement plus élevée que celle rencontrée chez les plantes non accumulatrices (Brooks et al., 1977). Aussi, selon Baker et Brooks (1989), les plantes hyperaccumulatrices devraient avoir une valeur seuil d'accumulation de métaux dans leur partie aérienne à 1% pour Zn, Mn, 0,1% pour Pb, Cu et Al, 0,01% pour Cd et Se et 0,001% pour Hg de leur matière sèche. Lombi et al., 2001 ont mis en évidence l'accumulation de 8,3 $mg.kg^{-1}$ de Cd et 200 mg.kg-1 de Zn par *Thlaspi caerulescens*, cette plante extrêmophile s'étant développée sur un sol contenant 19 $mg.kg^{-1}$ de Cd et 2920 $mg.kg^{-1}$ de Zn. Cependant, ces plantes qui tolèrent des concentrations 5 à 100 fois plus élevées que les autres métallophytes (Zn, Pb, Cd) ont une croissance lente et possèdent une faible biomasse. Par conséquent, un temps assez long est nécessaire pour décontaminer un site. De plus, l'accumulation d'un métal peut être modifiée quand on est en présence d'un sol contenant une contamination polymétallique. Actuellement, environ 400 espèces hyperaccumulatrices ont été répertoriées, on peut citer par exemple le tournesol, le colza, l'orge, le pissenlit (Baker et al., 1999).

III.2.1.2. La phytoextraction assistée (induite)

La phytoextraction induite se fait lorsqu'elle est assistée par des chélateurs de métaux qui sont appliqués au moment où la production de biomasse des plantes est optimale. Ces chélateurs vont stimuler l'accumulation de métaux dans la plante en changeant l'état chimique des métaux et former des espèces plus biodisponibles. Ces plantes sont caractérisées par leurs forte biomasse et à leur croissance rapide mais avec une faible capacité d'accumulation (Salt *et al.*, 1995).

La concentration de métaux dans les plantes est fortement influencée par la biodisponibilité de ces métaux dans le sol (Lasat, 2000). La biodisponibilité d'un métal dans le sol dépend des caractéristiques physiques et chimiques de la matrice, les espèces de métaux dans le sol, et de l'organisme biologique lui-même (Vangronsveld et Cunningham, 1998). Une fraction croissante biodisponible des métaux peut être un moyen d'améliorer l'efficacité de phytoextraction. Augmenter la quantité de minéraux disponibles dans le sol permet d'augmenter l'efficacité de la technique de phytoextraction. Ainsi, cette technique consiste à trouver le moyen d'augmenter la teneur métallique dans la plante par l'ajout d'agent chélatant (Huang et Cunningham, 1996; Blayblock *et al.*, 1997) ou d'engrais, par la sélection des types les plus accumulateurs au sein des populations naturelles ou encore par le transfert des gènes responsables de la capacité bioaccumulatrice (Raskin, 1996).

Les chélateurs des métaux comme l'EDTA (éthylene diamine tetra acetic acid) et EDDS (ethylene diamine disuccinate) pour le plomb, et l'EGTA (ethylene glyco- tetra acetic acid) pour le cadmium, sont connus pour améliorer la phytoextraction par l'augmentation de la biodisponibilité et de qugıenter l4extrqction des métaux lourds (Cooper *et al.*, 1999; Tandy *et al.*, 2006). Des expériences, réalisées par Jarvis et Leung (2002),

ont montré que la plante Pinus radiata n'est capable d'accumuler naturellement que 60 à 80 mg.kg-1 de plomb ; l'application d'un chélateur synthétique, comme l'EDTA ou l'H-EDTA multiplie par 5 ou 6 son pouvoir d'absorption du plomb (Jarvis et Leung, 2002). En fait, lors de l'ajout du chélateur dans le sol contaminé par le plomb, celui-ci est absorbé et migre vers la partie aérienne où il s'accumule sous la forme du complexe Pb-EDTA (Salt *et al.,* 1998 ; Huang et Cunningham, 1996). Ce phénomène a pu être décrit chez Zea mays L.; l'ajout d'EDTA ou d'EDDS permet une augmentation de l'accumulation de plomb dans la partie racinaire (Luo *et al.,* 2006) tandis que les engrais classiques sont une alternative prometteuse aux agents chélateurs pour améliorer la biodisponibilité, sans le risque supplémentaire de la contamination d'eau souterraine (Chaney *et al,* 1999). Une autre possibilité pour améliorer la biodisponibilité des métaux est l'utilisation de micro-organismes du sol ou les plantes dont les racines sont associées à des bactéries stimulées par les exsudats rqcinqores,i.e. un large éventail de molécules organiques (van der Lelie, 1998; Kamnev et van der Lelie, 2000)

III.2.1.3. Avantages et limites de la technique de phytoextraction

- **Les avantages**

La technique de phytoextraction présente de nombreux intérêts environnementaux et économiques. En effet, cette technique peut être appliquée sur des sites industriels (fonderies de métaux non ferreux, cokeries,…) et des sites agricoles (périphérie de sites industriels, épandage de déchets) afin de diminuer l'accumulation des métaux dans les sols, leur lessivage et leur transport par érosion. L'application de cette technique va aider à maintenir l'activité biologique et la structure des sols. De plus, le coût de la technique est bien moindre que celui de procédés traditionnels in situ et ex situ et varie selon les contaminants. Le coût d'un traitement in situ en présence de contaminants organiques et inorganiques est en effet de l'ordre de 10$ à 40$ par tonne de sols (Mulligan *et al.,* 2001). D'autre part, la phytoextraction est une des seules voies pour l'extraction sélective des métaux des sols, offrant de plus la possibilité de récupérer les métaux extraits. Les plantes utilisées permettent également de conserver un paysage agréable (reverdissement et floraison), et aussi d'installer une communauté de microflore et de microfaune métallo-résistante pouvant agir en synergie avec ces plantes afin d'accélérer le processus de décontamination. Les plantes utilisées peuvent être facilement récoltées et traitées (Kumar *et al.,* 1995; Raskin *et al.,* 1997; Garbisu et Alkorta, 2001). D'autre part, on peut aussi récupérer et réutiliser les métaux accumulés dans ces plantes. Enfin, en ce qui concerne l'impact sociétal, cette technique biologique est facilement acceptée par le public dans le contexte de la préservation de l'environnement, du développement durable, grâce à son impact esthétique lors de sa mise en œuvre.

- **Les inconvénients**

Comme toutes les techniques de phytoremediation, la phytoextraction comporte des inconvénients. Malheureusement, la majorité des plantes hyper-accumulatrices utilisées dans cette technique pour traiter les zones contaminées présentent une faible production de biomasse et une vitesse de croissance un peu longue ;

cela demande un investissement en temps, et/ou en argent assez important. La plupart des études ont pour objectif de découvrir des plantes hyperaccumulatrices ayant une forte production de biomasse dans un temps raisonnable. En plus, cette technologie s'applique uniquement à des sites faiblement ou moyennement contaminés car les plantes n'extraient qu'une quantité limitée de métal, au mieux quelques centaines de kg par an. Ce facteur limite la quantité de contaminants qui peut être extraite en un temps "raisonnable". Une autre limite est que le site à décontaminer doit présenter des conditions favorables au développement des espèces utilisées. De même, les facteurs édaphiques comme la granulométrie, le pH, les déficiences en éléments biogènes ou en oligo-éléments doivent être prises en compte.

III.2.2. La phytostabilisation

La phytostabilisation est une des techniques de phytoremediation la plus éprouvée. La phytostabilisation peut également être appelée reverdurisation ou revégetalisation. Cette technique de remédiation est particulièrement indiquée dans le cas de vastes zones contaminées caractérisée par une forte phytotoxicité du substrat (Bolan *et al.,* 2011 ; Pérez-de-Mora *et al.,* 2006, 2011; Domínguez *et al.,* 2008, 2009) et pour les quelles la contamination est trop importante pour pouvoir être traitée dans un délai raisonnable par phytoextraction. Cette technique n'est pas une méthode de dépollution au sens strict, mais elle permet d'immobiliser *in situ* les éléments traces métalliques et de diminuer les pools labiles des contaminants dans les sols. La phytostabilisation est réalisée grâce à l'utilisation de plantes qui ont la capacité d'adsorber les contaminants, ou de stabiliser le sol via le système racinaire, protégeant ainsi les sols de l'érosion par le vent et l'eau et réduisant les percolations d'eau à travers le sol. Cette technologie est également efficace pour empêcher la dispersion des contaminants dans les eaux de surface ou souterraines. Les plantes peuvent immobiliser les contaminants dans la zone racinaire par l'exsudation de composés chimiques, par l'inhibition des protéines de transport dans les membranes racinaires et par le stockage des contaminants dans les vacuoles des cellules racinaires (Bert et Deram, 1999 ; Anonyme, 2009). L'efficacité de cette technique peut être améliorée par l'application des amendements fertilisants et/ou stabilisants, organiques et/ou minéraux au sol avant la mise en culture (Vangronsveld *et al.,* 1995) pour accroître les qualités nutritives et diminuer la mobilité des métaux et la phytotoxicité du substrat. On parle alors de **phytostabilisation aidée** (Mench *et al.,* 2005, Vangronsveld *et al.,* 1995). Cette technique se décompose en deux étapes, l'immobilisation des contaminants et l'implantation de végétaux tolérants aux contaminants des sols. Les plantes utilisées en phytostabilisation doivent avoir certain caractères. Elles doivent (1) être tolérantes aux métaux du site à remédier, (2) être adaptées aux conditions climatiques et pédologiques du site, (3) avoir un système racinaire bien développé pour stabiliser efficacement le sol, (4) accumuler les métaux dans les racines et les transférer le moins possible dans les parties aériennes, afin d'éviter la contamination du couvert végétal, et (5) induire une importante couverture végétale (Berti et Cunningham, 2000).

Plusieurs types d'amendements peuvent précéder l'implantation des plantes tels que :

- des minéraux phosphatés;
- des amendements calciques;
- des aluminosilicates purs ou associés;
- des oxydes ou hydroxydes de Fe, Al et Mn;
- divers substrats organiques.

Les amendements changent la forme chimique des contaminants dans le sol et permettent la diminution de leur disponibilite et donc de leur toxicité (ADEME, 2010 ; Dechamp et Meerts, 2003).

Lorsque la contamination est localisée dans les premiers centimètres du sol, les meilleures plantes dans ces conditions pour la phytostabilisation sont généralement des graminées (pelouse) comme *Agrostis tenuis* (Vangronsveld *et al.*, 1995a; Vangronsveld *et al.*, 1995b), tandis que dans le cas de contaminations plus profondes, la meilleur façon pour traiter ce type de contamination est l'utilisation d'arbres et d'arbustes comme les peupliers et les saules, qui ont une réseau racinaire plus développé que les graminées. Au final, la mobilité du contaminant et son entrée dans la chaîne alimentaire sont réduites, et le couvert végétal va permettre d'accélérer le processus d'atténuation naturelle du sol (Wong, 2003).

III.2.2.1. Avantages et limites de la technique de phytostabilisation

➢Les avantages

La phytostabilisation est une technique récente. Seuls quelques essais de phytostabilisation ont été appliqués sur des sites contaminés pour tester l'efficacité de cette technologie à réduire la mobilité des métaux dans les sols (Mench *et al.* 2005; Peng & Yang 2005 ; Peng *et al.* 2005 ; Vangronsveld *et al.* 1996).

Les avantages de cette technologie est sa capacité à réduire la mobilité des éléments traces dans les sols et donc le risque des contaminants inorganiques sans les enlever de leur emplacement et à protéger/reconstructruire les sols car cette technologie ne génère pas de déchets contaminés qui nécessite un traitement secondaire. Elle est également capable de restaurer la couverture végétale par la restauration écologique de milieux fortement anthropisés. L'effet combiné des amendements et de l'installation d'un couvert végétal semble efficace et persistant dans le temps. Il faudra toutefois prévoir et maintenir un suivi de la dynamique des métaux dans le système sol-eau-plante après traitement. Cette technique s'applique principalement à des contaminations aux éléments traces métalliques. Elle constitue une technologie privilégiée pour les sites fortement contaminés. La manutention associée à cette écotechnologie par rapport à d'autres technologies d'assainissement comme l'excavation est limitée (semblable à celle des procédés agricoles), et les coûts sont généralement plus bas. Toutefois, les coûts réels dépendent des conditions particulières au site (profondeur de la contamination, l'état des sols, le besoin de conditionnement du sol et le labourage).

➢Les inconvénients

Cette technologie souffre de plusieurs limites telle que :

1- Les contaminants ne sont pas extraits des sols et laissés en place sous des formes peu ou pas mobiles éventuellement mobilisables par des modifications de conditions physico-chimiques des sols

2- Il faut choisir aussi des plantes qui peuvent stocker préférentiellement les métaux dans leurs racines, ce sont les « excluders » (Masarovičová *et al.*, 2010).

3- Si les concentrations de contaminants sont élevées, les effets toxiques peuvent empêcher les plantes de se développer jusqu'à la réduction de leur biodisponibilité pour les plantes.

4- Si des amendements de sol sont utilisés, ils peuvent avoir besoin d'être réappliqués régulièrement pour maintenir l'efficacité de l'immobilisation des contaminants dans les sols.

Chapitre1.3.

Modélisation mathématique dans le domaine des sols contaminés

I. Introduction

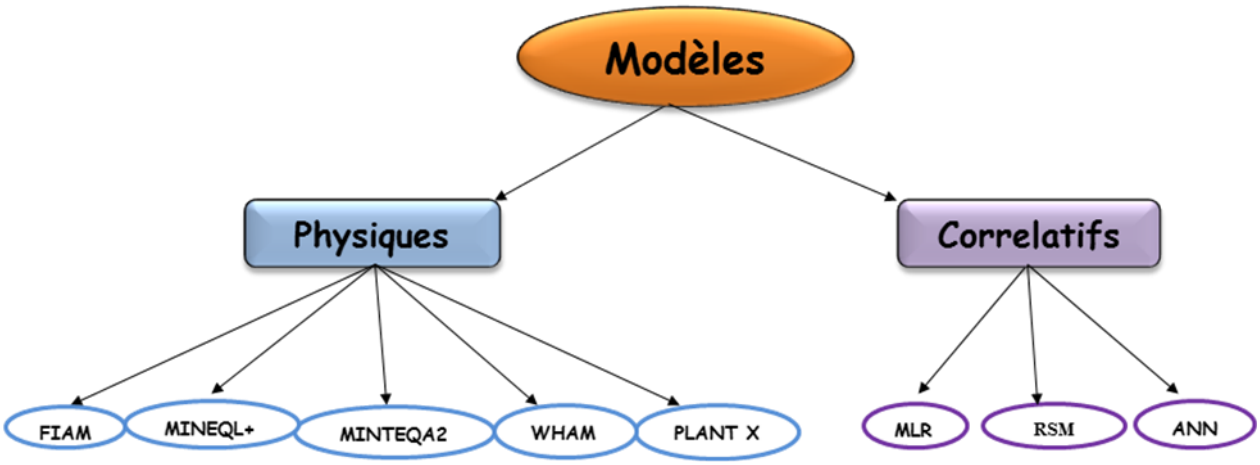
L'objectif du chapitre est de donner une brève présentation sur les principales approches de modélisation mathématique dans le domaine des sciences du sol. Les principales approches seront décrites et les limitations des modèles seront présentées. L'accent sera mis sur la description de la modélisation par des réseaux de neurones artificiels, approche utilisée dans le cadre de la thèse.

En effet, prédire l'évolution des systèmes géochimiques et les effets associés aux évolutions sur la mobilité et la disponibilité ainsi que le transfert des éléments traces depuis les sites et sols contaminés vers les végétaux est un de défi majeur (Wagenet et Rao, 1990). Par conséquence, une caractérisation des transferts de substances polluantes dans les sols est indispensable pour réduire les incertitudes liées aux évaluations (Minasny and McBratney, 2002 ; INERIS, 2002). Pour atteindre cet objectif, la modélisation est un outil très utile qui peut être utilisé pour acquérir une plus grande compréhension des processus physico-chimiques à la fois à interpréter les expériences en laboratoire et les données de terrain ainsi que d'effectuer des prédictions à long terme des comportements géochimiques (Brown and Allison, 1987; Banton et Villeneuve, 1989 ; Lindström, 2005).

Plusieurs modèles de simulation ont été initialement appliqués à la compréhension de questions fondamentales dans les milieux aquatiques (Minasny *et al.*, 2004 ; Twarakavi *et al.*, 2008), les problèmes de pollution des eaux de surface (Lafrance et Banton 1995), et l'évaluation des processus diagénétiques (impliquant la formation naturelle et l'altération des roches), l'analyse des problèmes environnementaux impliquant les eaux souterraines, l'exploitation minière, le stockage géologique des déchets et les déversements chimiques (Stenemo *et al.*, 2005 ; Parkhurst and Appelo, 1999).

II. Modélisation mathématique appliquée aux sols

Les modèles les plus utilisés dans le domaine de l'environnement peuvent être classés en deux grandes familles: les modèles physiques et les modèles corrélatifs (Fig. 10). Chaque famille peut être déclinée à son tour en un certain nombre d'approches spécifiques.



FIAM : Free Ion Activity *Model*; **MINEQL +:** A chemical equilibrium modelling system; **MINTEQA** 2: Modèles d'évaluation de l'exposition; ***WHAM*** : Windermere Humic-Aqueous *Model;* ***PLANT X****:* Soil-planet-air transfer model; **MLR**: Régression multiple linéaire; **RSM**: Méthode des surfaces des réponses ; **ANN** : Réseaux de neurones artificiels

Figure 10. Exemple de modèles physiques et corrélatifs utilisé dans le domaine de l'environnement.

Dans la suite, une bibliographie relative aux modèles physiques et corrélatifs sera donnée. Ceci permet (i) à un lecteur non spécialiste de trouver des éléments de réflexions pour construire un modèle mathématique adapté à son problème de sol et (ii) justifier la modélisation retenue dans le cadre de cette thèse basée sur les réseaux de neurones artificielles.

II.1. Les modèles physiques

Les modèles physiques sont des modèles de nature déterministe qui prennent en compte et décrivent les mécanismes physiques intervenant dans la transformation des entrées (causes) du problème en réponses (effets) (Fig. 11). C'est l'approche qui requiert le moins de ressources informatiques puisqu'elle cherche à approcher le phénomène et/ou la structure étudiés de façon à avoir le modèle le plus représentatif possible. Pour cela, les modèles physiques déterministes se basent sur les équations analytiques issues d'hypothèse et de considérations théoriques régissant un phénomène et les étudient sur une représentation abstraite du système réel, prenant en compte tout ou partie du phénomène analysé.

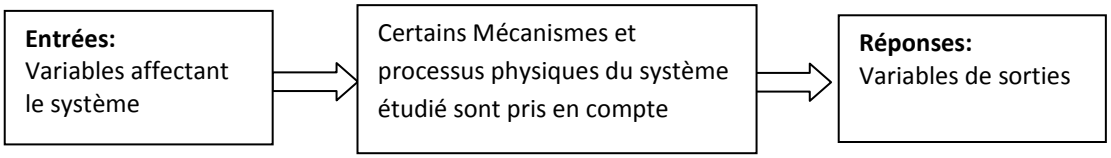


Figure 11. Principe d'un modèle physique.

Cependant avant de sélectionner un modèle physique pour décrire le problème étudié, il faut bien choisir le modèle représentatif du phénomène étudié et les variables d'entrées et sorties correspondantes. En particulier, les interactions des matières organiques avec les métaux dans les sols sont dominantes dans le milieu naturel.

Dans ce cas, lorsque le cadre théorique est connu et le modèle défini, on est capable de prédire les variables de sorties représentant la réponse du phénomène étudié.

Dans le domaine du sol, pour étudier la spéciation des élémenst traces dans le sol, plusieurs modèles physiques ont été utilisés soit pour analyser la distribution des métaux parmi les espèces aqueuses soitentre la phase aqueuse et la phase solide. Parmi ces modèles on peut citer MINTEQA2 (Allison *et al.*, 1991) et MINEQL+ (Schecher and McAvoy, 2001) et PHREEQ (Parkhurst *et al.*, 1980). Ces modèles peuvent être utilisés pour calculer les activités chimiques et les concentrations aqueuses de l'ion métallique libre ainsi que des complexes métalliques inorganiques. D'autres modèles ont été développés pour prédire l'absorption des éléments traces dans les sols, les organismes et leurs effets toxiques sur les êtres vivant des sols. Le premier modèle utilisé dans le milieu aquatique est connu sous le nom de « Free Ion Activity Model » (FIAM, Morel, 1983). Il est basée sur les mécanismes chimiques sous-jacents aux processus d'absorption (van Leeuwen, 1999 ; Lofts *et al.*, 2005). Il pose que la biodisponibilité des métaux dans les systèmes aqueux est controlée par l'activité ionique de métaux libres plutôt que par la concentration totale ou dissoute. Ceci est dû au fait que les ions métalliques libres (par exemple, Cu^{2+}) sont facilement absorbés par les organismes, tandis que les métaux particulaires et fortement complexés ne le sont pas. Si le modèle FIAM ne s'intéresse qu'aux formes métalliques libres en solution, la « pore water hypothesis » considère tous les ETM en solution, incluant les formes dissoutes et complexées à des ligands organiques ou inorganiques. Ce type d'approche apparaît comme un outil pragmatique présentant des capacités prédictives de la bioaccumulation chez plusieurs organismes du sol (van Gestel, 1997 ; Peijnenburg *et al.*, 1999).

Un autre modèle, PLANT X, a été développé par Trapp et McFarlane en 1995, pour étudier les transferts des polluants organiques des sols vers quatre compartiments végétaux supposés homogènes: racine, tige, feuille et fruit. Les processus de transfert plante/environnement ainsi que les processus internes à la plantequi sont considérés par la modèle sont les suivants:

- prélèvement du polluant depuis le sol et translocation vers les feuilles
- de transpiration;
- échanges gazeux entre les feuilles et l'atmosphère;
- translocation avec le courant d'assimilation vers les fruits depuis les feuilles ;
- transformation et dégradation (métabolisme);
- dilution du polluant par croissance de la plante.

Egalement, plusieurs modèles de spéciation chimique considérant les réactions des métaux traces avec les matières organiques naturelles des sols ont été développés. Le modèle Windermere humique aqueuse - Modèle V (WHAM V, Tipping, 1994) a été l'un des premiers modèles permettant de calculer la spéciation chimique d'équilibre dans les eaux de surface, les eaux souterraines, les sédiments et les sols contenant de la matière organique naturelle (NOM).

Pour pallier aux limitations des modèles physiques, des approches empiriques de type causes à effets

basées sur la corrélation mathématique des variables de sorties en fonctions des variables d'entrées ont été développés. Ces approches n'intègrent pas la description des mécanismes physiques caractérisant les réponses et n'expliquent pas les processus en cours ; cependant ils offrent la possibilité de prédire les réponses en fonction des entrées d'une façon relativement fiable et robuste.

II.2. Les modèles corrélatifs

Les modèles corrélatifs sont particulièrement adaptés aux cas où une première estimation de l'effet potentiel d'un ou plusieurs paramètres est nécessaire, en particulier lorsque les détails et les mécanismes réactionnels ne sont pas bien connus (Fig. 12).

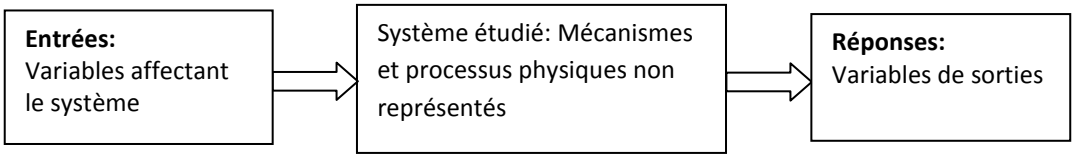


Figure 12. Principe d'un modèle corrélatif de type causes à effets sans description des mécanismes et processus physiques se produisant dans le milieu.

Ces modèles ont été développés pour améliorer la compréhension des processus pédologiques et d'agir également comme des outils pour évaluer les problèmes agricoles et environnementaux. Par conséquent, les modèles de simulation sont régulièrement utilisés dans la recherche et la gestion (Minasny and McBratney, 2002).

On distingue principalement deux types de modèles : la régression multiple linéaire (MLR) et les surfaces de réponses (RSM).

II.2.1. La méthode de régression linéaire multiple (MLR)

La méthode la plus couramment utilisée dans les MLR consiste à employer des régressions linéaires multiples (Fig. 13). La régression linéaire multiple cherche à approximer une relation fonctionnelle souvent complexe en général, par une fonction mathématique simple par une équation de la forme (Elzinga *et al.*, 1999; Schug *et al.*, 2000; Römkens and Salomons, 1998; Tiktak *et al.*, 1998):

$$y = \alpha_0 + \sum_{i=1}^{n} \alpha_i x_i \qquad (1)$$

y est la sortie, x_i sont les variables d'entrées, α_0 et α_i sont les paramètres de l'équation.

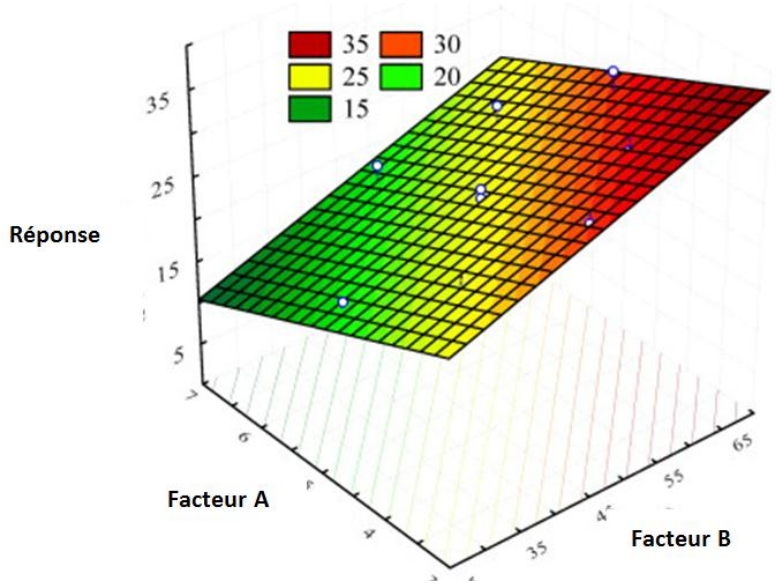


Figure 13. Illustration d'une réponse MLR.

II.2.2. La méthode des surfaces de réponses (RSM)

Comme alternative aux techniques de régression linéaire, la méthode des surfaces de réponses présente l'avantage de modéliser les réponses non linéaires d'ordre 2. La méthode des surfaces de réponses (RSM) (Fig. 14) a pour but d'établir les relations (mathématiques) entre les variables d'entrée et de sortie simulant le comportement d'un phénomène à partir d'un certain nombre d'expériences. Une réponse y est reliée à un ensemble de paramètres d'entrée x_i par le biais d'une fonction de type $y = f(x_i)$. L'idée principale de la méthode est d'utiliser un modèle mathématique non linéaire (polynôme de second degré) pour approximer la relation entre les entrées et les sorties sous la forme (Onur and Necip, 2003 ; Cornell, 1990 ; Hambli *et al.*, 2005):

$$y = \beta_0 + \sum_{i=1}^{n} \beta_i x_i + \sum_{i=1}^{qn} \beta_{ii} x_i^2 + \sum_{j=1}^{q} \sum_{i=1}^{n} \beta_{ij} x_i x_j, \qquad (i < j) \qquad (2)$$

x_i et x_j sont les variables d'entrées et β_0, β_{ii} et β_{ij} sont les paramètres de l'équation.

Cette méthode s'applique typiquement après une étude de screening pour utiliser uniquement les facteurs les plus influents.

La RSM devient alors un outil d'investigation pour étudier le comportement du système. Pour un grand nombre de facteurs, la mise en œuvre de la RSM devient délicate en raison des éventuels interactions multiples entres les entrées. Des outils mathématiques spécifiques peuvent être utilisés à ces fins.

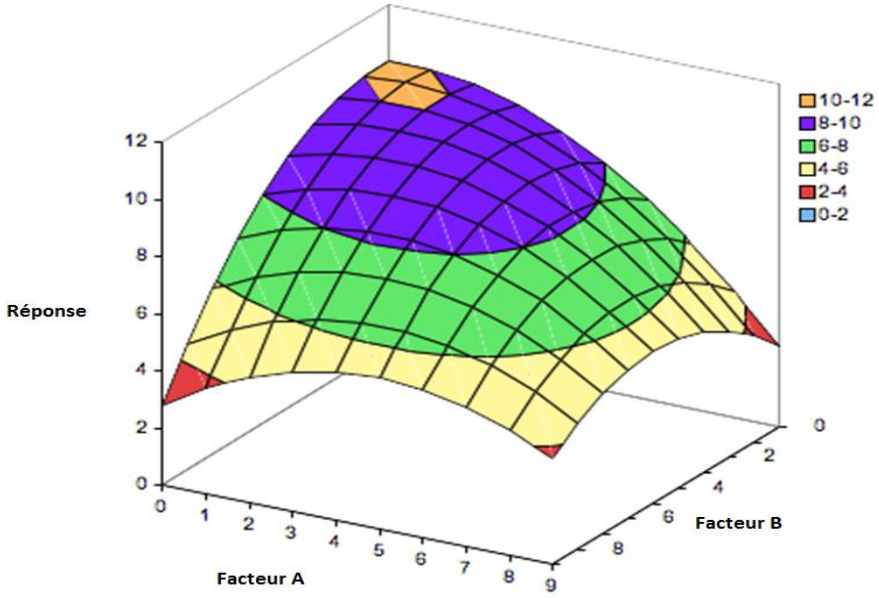


Figure 14. Illustration d'une réponse RSM.

II.3. Démarche pour construire un modèle mathématique en 3 étapes

La construction d'un modèle corrélatif décrivant la réponse d'un sol nécessite de suivre une démarche séquentielle en 3 étapes :

- Choix des facteurs et du domaine expérimental (screening).
- Réalisation d'essais selon un plan d'expériences spécifique.
- Construction mathématique du modèle à partir des résultats du plan d'expériences.

II.3.1. Choix des facteurs et du domaine expérimental (screening)

Pour garantir la fiabilité de prédiction d'un modèle corrélatif, il faut obligatoirement procéder à la sélection des variables d'entrées capables de capter le maximum d'informations conduisant à une prédiction précise et fiable. Dans le cas où il y a un grand nombre de facteurs, un criblage (*screening)* (Watson, 1961; Kleijnen, 1975; Bettonvil, 1995) doit permettre de détecter les facteurs les plus influents et leur domaine de variation. Elle constitue une étape importante avant toute analyse plus fine ultérieure.

Dans le cas des modélisations liées aux sols, le problème est d'identifier parmi tous les facteurs d'entrée mesurables ceux qui ont une influence importante sur la réponse du sol. Une étude de criblage (screening) des paramètres opératoires par la méthode de plans d'expériences, afin de déterminer leurs niveaux optimaux et aussi d´éliminer ceux qui ont peu d'influence (Winer *et al.*, 1991; Underwood, 1997, 1981; Mead, 1988). Ceci permet un gain de temps considérable en termes d'expérimentation et l'augmentation de la fiabilité des résultats.

L'analyse d'un processus expérimental doit commencer par une identification des principales causes susceptibles d'influencer d'une manière significative les résultats des essais. Parmi les facteurs recensés par l'expérimentateur, la technique de criblage permet de déterminer ceux qui ont une influence statistiquement non négligeable sur la variation de la réponse. Cet outil nous permet aussi une simplification du problème (débroussaillage) (Seong *et al.*, 2003; Benoist *et al.*, 1994 ; Schimmerling *et al.*, 1998).

A l'aide de cette technique on réalise la comparaison entre les variations de la réponse engendrée par deux sources différentes :

1. les variations des facteurs étudiées
2. les variations de grandeurs considérées comme n'ayant pas d'effet sur la réponse (facteurs bruit).

Un facteur est jugé influent (ou d´exterminant) si son action sur la réponse étudiée est statistiquement (l'analyse de la variance) supérieure à un certain niveau fixé par l'expérimentateur. Ainsi, cette analyse permet de détecter et de hiérarchiser les influences des actions d'un modèle (Benoist *et al.*, 1994). Généralement pour l´étude de screening (Mathieu et Phan-Than-Luu, 2001) on utilise des plans factoriels complets tant que le nombre de facteurs n'est pas très grand, mais il est possible d'utiliser le plan le mieux adapté à la problématique étudiée. Ces plans ont été largement publié (Montgomery, 2001; Pillet, 1997 ; Dean, 2000) et sont très faciles à utiliser.
Le modèle mathématique utilisé dans toute étude de criblage est donc un modèle polynomial de premier degré.

II.3.2. Plan d'expériences (PEX) pour la réalisation des essais

La construction d'un modèle corrélatif se base principalement sur les résultats d'essais associant des entrées à des sorties. Pour optimiser la construction d'un modèle et l'identification de ses paramètres, il est indispensable de réaliser les essais selon des procédures particulières (nombre d'essais et combinaisons entre les facteurs) organisés au préalable selon des plans d'expériences (Goupy, 2001, 1999). Ces plans d'expériences sont applicables à de nombreuses disciplines et à tous types d'essais à partir du moment où on cherche à trouver le lien qui existe entre une grandeur d'intérêt (sorties, réponses) y, et des variables (facteurs), x_i. Avec les plans d'expériences on obtient le maximum de renseignements avec le minimum d'expériences. Pour cela, il faut suivre des règles mathématiques et adopter une démarche rigoureuse pour obtenir les meilleures réponses (Box *et al.*, 2005). Le succès de la démarche originale des plans d'expériences réside dans la possibilité de diminuer le nombre d'essais, d'étudier un grand nombre de facteurs et de détecter les interactions entre ces facteurs avec une précision optimum des résultats. En plus la mise en œuvre d'un PEX permet un gain en temps et une réduction des coûts liés aux expérimentations. Un PEX, correspondant à la réalisation d'une série de N expériences, qui sont toutes : déterminées *a priori* ; obligatoirement réalisables et indépendantes. Par conséquent pour

comprendre comment réagit un système en fonction des facteurs susceptibles de le modifier, il faut mesurer une réponse et ensuite essayer d'établir des relations de cause à effet entre les réponses et les facteurs. Parmi les facteurs on distinguera plusieurs types:

- les facteurs contrôlables qui dépendent directement du choix du technicien (pression, température, matériau,….)
- les facteurs non contrôlables qui varient indépendamment du choix du technicien (conditions climatiques, environnement d'utilisation…)

Le PEX peut s'appliquer a tous les phénomènes type boîte noire (Fig. 15) ou l'on cherche à optimiser les données de sortie (les réponses) en réglant les données d'entrée (les facteurs). Les données d'entrée sont connues dans la littérature comme étant des facteurs qui peuvent être contrôlées, mais il existe parfois des facteurs qui ne peuvent pas l'être, par conséquent ils ont reçu l'appellation de facteurs bruits.

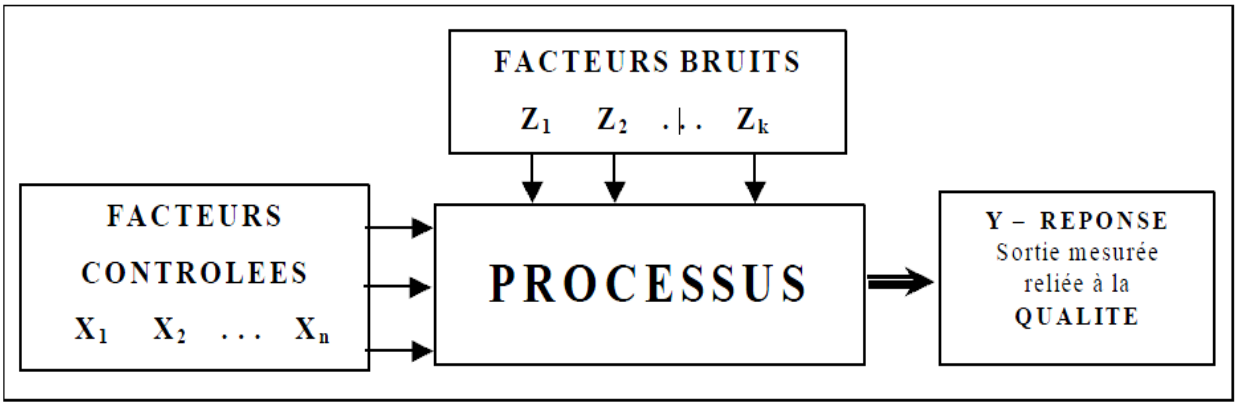


Figure 15. La boîte noire du processus.

En résumé, les objectifs des plans d'expériences sont de:

–diminuer le nombre d'essais (ou de calculs).

–étudier les effets des paramètres.

–déduire les paramètres influents.

–evaluer les interactions entre facteurs.

–avoir une meilleure précision sur les résultats.

–établir une modélisation mathématique de la réponse.

Dans le cas où les facteurs sont contrôlables, le choix du plan d'expérience va dépendre de la nature de la réponse obtenue. Si on sait que la relation entre les facteurs et les réponses sont de type linéaire on peut appliquer un plan d'expérience à deux niveaux comme le plan factoriel complet à deux niveaux ou le plan factoriel à K facteurs, à 2 niveaux également.

On distingue essentiellement les plans d'expériences suivants:

– plans de criblage (screening): pour trouver les facteurs les plus influents sur une réponse.

– plans de modélisation = plans pour surfaces de réponse : modèles du 1er ou 2ème degré

– plans de mélanges : adaptés aux facteurs dépendants

– plans complets/fractionnaires :

– plans factoriels complets : toutes les combinaisons des niveaux de facteurs sont présentes
– plans factoriels fractionnaires : tous les niveaux de chaque facteur sont présents, mais pas toutes les combinaisons possibles de facteurs

Les plans d'expériences les plus simples utilisent seulement 2 niveaux (réponse linéaire), pour les systèmes non-linéaires, un plan factoriel complet avec 3 niveaux par facteur s'impose mais généré un très grand nombre à réaliser. En contrepartie, il permet une meilleure représentation du problème étudié.

II.3.3. Construction mathématique d'un modèle.

Le choix d'un modèle (MLR : linéaire ou RSM : non-linéaire) dépend du type de problème étudié. A partir des valeurs des réponses, y, obtenues dans le plan d'expériences**,** on ajuste un modèle que l'on souhaite prédictif. Ce modèle doit être rapide à ajuster, notamment en grande dimension, et facile à interpréter. Ces résultats peuvent servir pour établir plusieurs modèles corrélatifs comme MLR: des modèles linéaires, RSM: surface de réponses (comme les MLR mais non linéaire) et les réseaux des neurones artificiels (ANN). On va développer plus tard les modèles de MLR et ANN utilisés dans notre travail.

De point de vue technique, la construction proprement du modèle se fait habituellement à l'aide d'un logiciel de statistique de type Statistica ou autres.

II.4.Les réseaux de neurones artificiels (ANN)

Un réseau de neurones artificiels (ANN) est un modèle de calcul dont la conception est très schématiquement inspirée du fonctionnement du cerveau et du système nerveux humain. Un ANN 'apprend' lors d'une phase d'apprentissage à associer des sorties à des entrées issues de résultats d'expériences (Corsi et Noël, 1990; Putter et Smeets, 1989; Sejnowski et Tesauro, 1989; Behrens *et al.*, 2005; Gandhimathi and Meenambal, 2012). Il construit ainsi un 'savoir' sous forme d'un modèle mathématique sous forme de connexions entre un certain nombre de neurones artificiels. Les entrées circulent dans des calculs intégrant les facteurs les plus influants. Il sera capable par la suite de prédire le comportement du système (sorties) pour des combinaisons d'entrées non présentées lors de l'apprentissage (Römkens and Salomons, 1998; Tiktak *et al.*, 1998; Schaap and Lei, 1998; Elzinga *et al.*, 1999; Sarmadian and Taghizadeh Mehrjardi, 2008).

II.4.1. Structure d'un ANN

La famille de réseaux des neurones la plus utilisée est le perceptron multi-couches (PMC). L'architecture de cette famille d'ANN est composée (Fig. 16) (Bishop, 1995; Haykin 1996; Thiria *et al.*, 1993):

- d'une couche d'entrée (contenant les variables d'entrées xi) composée de Ni neurones,

- un certain nombre de couches cachées prenant leurs entrées sur les $Ni - 1$ neurones de la couche précédente
- et une couche de sortie(s) (contenant les variables de sortie(s) yi) dans les connexions vers l'avant.

Chaque neurone dans la couche d'entrée représente un seul paramètre d'entrée. Puis cette couche transfert ces valeurs aux couche suivantes cachées ; finalement les neurones de la dernière couche représentent les sorties ANN. La « couche » d'entrée n'est pas une réelle couche de neurones car elle se contente de coder les variables d'observation. La couche de sortie code la variable de discrimination. Les valeurs d'activité des neurones sont propagées dans le réseau, de l'entrée vers la sortie, sans retour arrière. La présence d'une couche cachée permet de modéliser des relations non linéaires entre les entrées et la sortie. Les neurones sont reliés entre eux par l'intermédiaire de poids synaptiques, notés W_{ij}.

Cependant l'utilisation d'un ANN se fait en trois phases (Haykin, 2009):

(i)une phase d'apprentissage

(ii)une phase de validation

(iii)une phase d'utilisation.

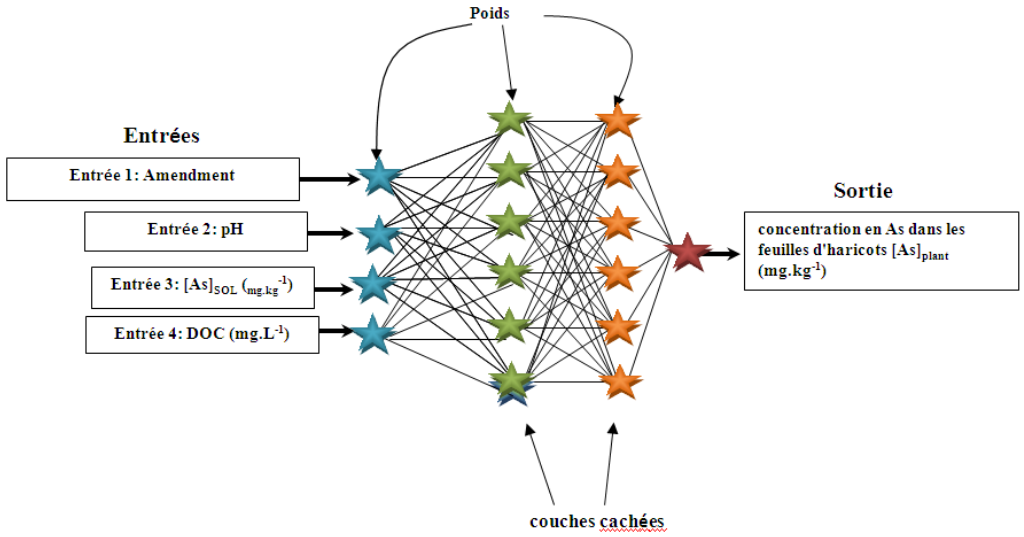


Figure 16. Architecture d'un réseau de neurones composé de 4 entrées, deux couches cachées et une couche de sortie. Exemple de modélisation de la phytoextraction : concentration d'As dans les feuilles de haricot (sortie) en fonction des caractéristiques principales du sol contaminé en As p (entrées).

Malgré la supériorité des ANN par rapport aux MLR et RSM, peu d'études existent dans la littérature mettant en œuvre la méthode des ANN pour modéliser des réponses relatives à des problématique de sols contaminés à partir de paramètres facilement mesurables telles que le pH, la granulométrie (teneur en sable, de limon et d'argile), la teneur en matière organique, la densité apparente et la porosité. Les articles suivant ce chapitre contiennent chacun une étude bibliographique sur le sujet.

II.4.2. L'apprentissage

L'apprentissage d'un réseau de neurones artificiels est induit par une procédure itérative d'ajustement de ses paramètres internes (poids synaptiques et nombres de neurones). Ces ajustements sont décrits par un algorithme d'apprentissage.

Il existe deux types d'apprentissage, supervisé et non supervisé. Dans le domaine de modélisation/prédiction par ANN, l'apprentissage supervisé est le plus couramment utilisé.

Le processus de l'apprentissage de l'ANN consiste à présenter un ensemble d'exemples (entrées avec des sorties connues résultats d'essais par exemple) (Jenkins, 1997; Rafiq *et al*., 2001, Hambli *et al*., 2006, Hambli, 2009). La phase d'apprentissage est chargée d'ajuster les valeurs des poids de connexions du réseau (W_{ij}). L'algorithme d'apprentissage aura pour tâche d'évaluer ces poids en fonctions des données présentées lors de la phase d'apprentissage. Lors de cette phase, l'objectif principal de l'ANN est de trouver d'une façon itérative la meilleur configuration de l'ANN en minimisant l'erreur entre les valeurs des sorties présentées lors de l'apprentissage et les valeurs des mêmes sorties prédites lors des boucles d'apprentissage. Ce savoir est stocké sous forme de poids de connexions (W_{ij}) entre les différentes couches de neurones (entrées-cachées-sorties). Une fois le cycle d'apprentissage (epochs) terminé, l'architecture de l'ANN garde et utilise les poids (W_{ij}) ayant assurés l'erreur minimale entre prédictions/mesures lors de l'apprentissage.

A chaque itération une entrée est présentée au réseau, il propage ces valeurs vers les neurones de sortie. Cette sortie calculée est comparée avec la réponse désirée, s'il y a des déférences, le système utilise ces différences pour adapter les paramètres du réseau interne de façon à corriger son comportement et donner les valeurs désirées en conséquence. Cette altération des connexions est obtenue par l'algorithme de "rétro propagation" du gradient d'erreur. Ce calcul est chargé de rétro propager dans le réseau les erreurs constatées sur les sorties. Finalement cette connaissance est représentée et stockée par la force (poids) des connexions entre les neurones (Fig. 17).

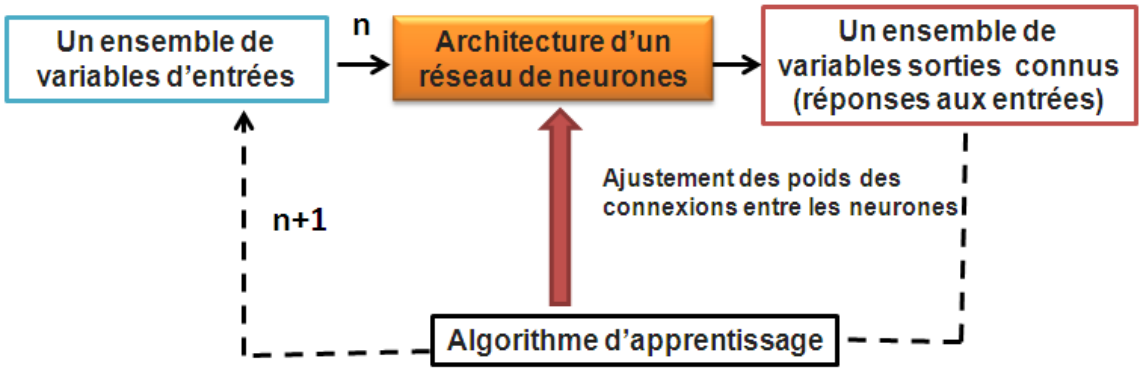


Figure 17. Illustration de la phase d'apprentissage qui consiste à ajuster les poids de connexion entre les neurones à l'aide d'un algorithme de rétro-propagation. L'apprentissage est répété un certaine nombre de cycle (epochs) pour atteindre une faible erreur quadratique moyenne ou RMSE (Root Mean Square Error).

Il existe plusieurs algorithmes d'apprentissage pour ANN. Les plus utilisés sont basés sur des algorithmes de rétro-propagation de type Levenberg-Marquardt (Jenkins, 1997; Rafiq *et al*., 2001 ; Hambli *et al*., 2006 ; Hambli, 2009).

Dans l'apprentissage non supervisé, l'utilisateur du réseau ne détermine pas de variables de sortie et n'interviens pas dans la fonctionnalité du système. Le réseau s'auto-organise de façon à découvrir des formes récurrentes dans les informations qu'il reçoit, mais en manière indépendante sans aide extérieures. Cet apprentissage est destiné à l'élaboration d'une représentation interne de l'espace des données d'entrée en identifiant la structure statistique sous-jacente des variables d'entrée sous une forme plus simple ou plus explicite.

II.4.3. Validation d'un modèle ANN

Une fois l'apprentissage terminé, il faut vérifier que le modèle ANN modélise/prédit correctement les réponses. La validation consiste à présenter des résultats d'essais (entrées-sorties) non présentés lors de la phase d'apprentissage et de vérifier si la prédiction du ANN est en proche des valeurs mesurées.

II.4.4. Utilisation du modèle ANN

Une fois le modèle validé, on peut utiliser l'ANN comme outil de prédiction où l'on présente au réseau de nouvelles combinaisons d'entrées et l'ANN prédit en retour les sorties.

II.5. Modélisation déterministe et stochastique

L'approche de modélisation déterministe considère que les entrées ont chacune une valeur fixe possédant pas une certaine incertitude ou variabilité statistique par rapport à la valeur de référence. L'approche de modélisation stochastique généralise la démarche en considérant que les mesures (entrées) possèdent une variabilité due à des causes de type incertitude de mesures, variation/variabilité statistique naturelle. On entendra par variabilité, le fait qu'un système fluctue pour une cause inconnue ou non contrôlée. Le caractère flou que nous pouvons avoir d'un paramètre peut avoir deux origines : la variabilité, conséquence intrinsèque du phénomène étudié qui n'est pas fixe, et l'incertitude, entraîné par une information insuffisante vis à vis de ce paramètre. Il est bien entendu fondamental, bien que pas forcément aisé, de distinguer les deux sources. Une modélisation déterministe ne fait intervenir aucune variation des variables d'entrées. La variabilité n'est pas modélisée par simulation. Une modélisation stochastique fait intervenir les variations statistiques des variables d'entrées. La variabilité est donc modélisée par simulation.

La simulation de modèles stochastiques nécessite le recours à une répétition des calculs présentant à chaque fois les entrées générées avec une distribution aléatoire selon une loi statistique (normale, log-normale, *etc…*). Ce type de calcul peut être effectué selon la méthode de Monte-Carlo (par référence aux jeux de hasard

des casinos). La méthode de Monte Carlo est une méthode numérique, qui utilise des tirages aléatoires pour réaliser le calcul d'une quantité déterministe.

II.6. L'intérêt de l'utilisation des ANN

Dans la pratique, le problème qui se pose est de trouver une relation entre un ensemble de sorties dans un processus donné, et l'ensemble des entrées correspondant, à travers les mesures effectuées. En d'autres termes, l'ingénieur cherche un modèle du processus qu'il étudie à partir des mesures dont il dispose : on dit qu'il effectue une modélisation de type "boîte noire" (Pernot and Lamarque, 1999).

La supériorité des ANN se justifie essentiellement à partir des limitations des modèles corrélatifs de type MLR et RSM qu'on peut résumer comme suit:

- les modèles MLR et RSM nécessitent de proposer une forme d'équation *a priori* chose qui n'est pas toujours facile à faire par un non spécialiste.
- ils ne permettent pas de modéliser des entrées de type attributs (bon, moyen, sol1, sol2, amendements1, 2, ...).
- ils ne permettent pas d'effectuer des calculs inverses : prédire des entrées corresponds à une réponse cible donnée.
- ils sont très couteux en temps de calcul pour effectuer du calcul stochastique.

En revanche:

- la mise en œuvre d'un ANN ne nécessite pas de connaissances *a priori,* relatives au comportement du processus étudié.
- le développement d'un modèle ANN (architecture) est simple et rapide pour un non spécialiste.
- les ANN permettent une analyse stochastique du problème de type Monte Carlo extrêmement rapide à cause du calcul parallèle offert par les ANN.
- les coefficients de corrélation (prédiction/essais) dans le cas des calculs par ANN sont généralement supérieurs à ceux obtenus par MLR et RSM.

De manière générale, un réseau de neurones permet de faire un meilleur usage des mesures disponibles que les méthodes d'approximation conventionnelles (MLR, RSM) (Mozolin *et al.*, 2000). Ce gain peut être considérable lorsque le processus à modéliser dépend de plusieurs variables comme dans le cas des sols où de nombreuses interactions non-linéaires peuvent se produire.

II.7. Logiciel NeuroMod et développement du module 'Soil Pollution Prediction'

Il existe un certain nombre de logiciels commerciaux d'ANN. On peut citer par exemple les modules ANN de Statistica et de Matlab. Dans le cadre de cette thèse, un programme d'ANN développé précédemment

en Fortran baptisé Neuromod (Hambli *et al.*, 2006; Hambli *et al.*, 2009) a été adapté pour simuler les problèmes de sols (Hattab et al., 2013) ((Fig. 18).

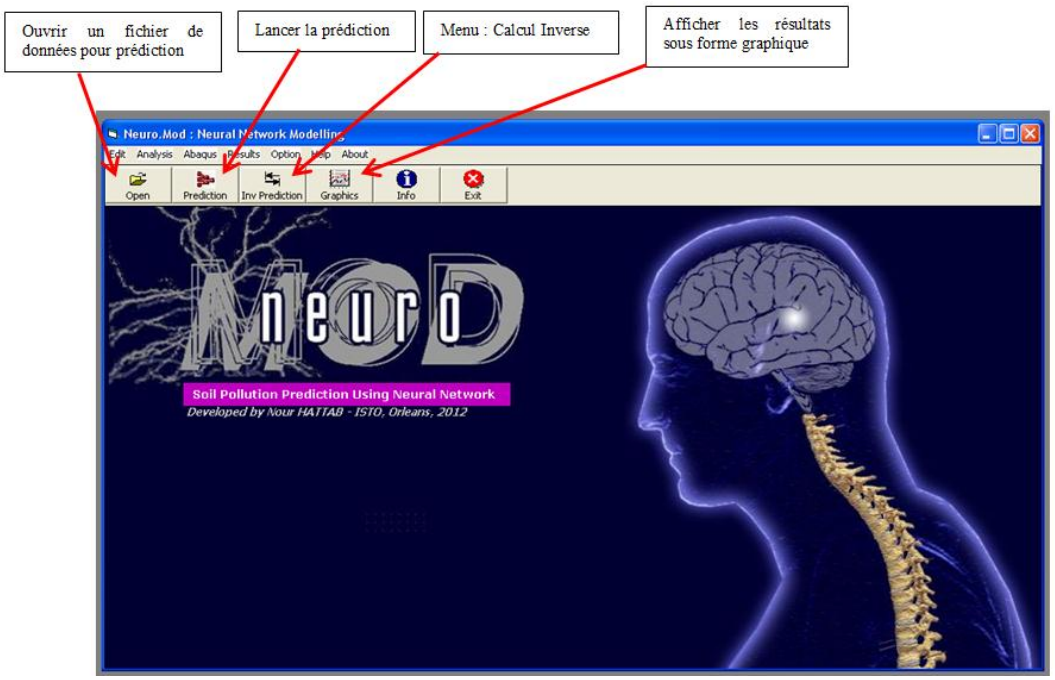


Figure 18. Interface du Logiciel Neuromod adapté pour modéliser les problèmes de contaminations des sols dans le cadre de la thèse.

Une interface graphique conviviale a été développée sous l'environnement Visual Basic 6 (BV6).

Quatre menus ont été programmés permettant une utilisation très simple et immédiate du Module 'Soil Pollution Prediction' (SPP):

1. Ouverture et lecture du fichier contenant les entrées pour prédiction en format *.txt (Fig. 19).

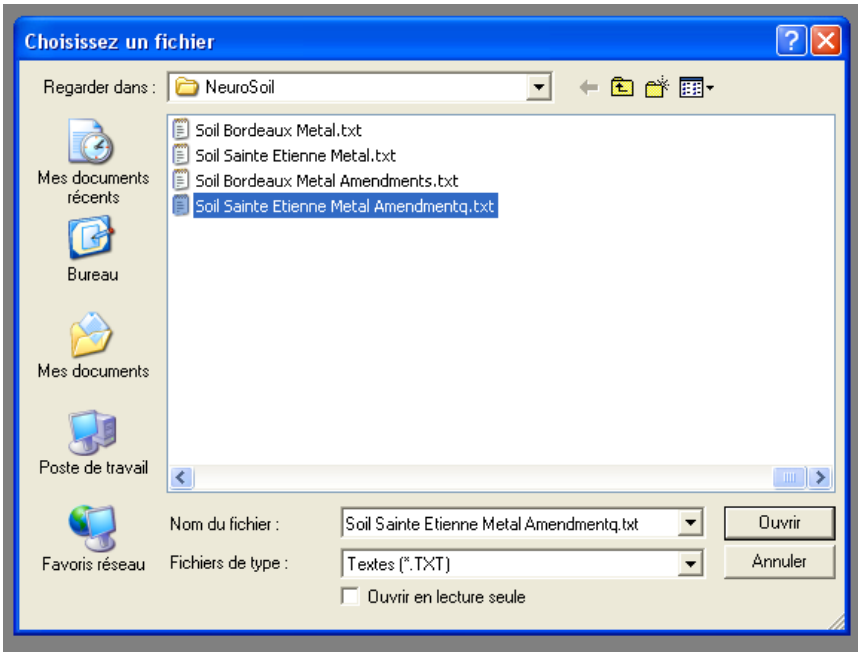


Figure 19. Module « Soil Pollution Prediction » du logiciel Neuromod. Menu permettant d'ouvrir un fichier de données expérimentales pour effectuer une prédiction.

2. Lancement des calculs et affichage des résultats sous forme de tableaux de type Excel (Fig. 20).

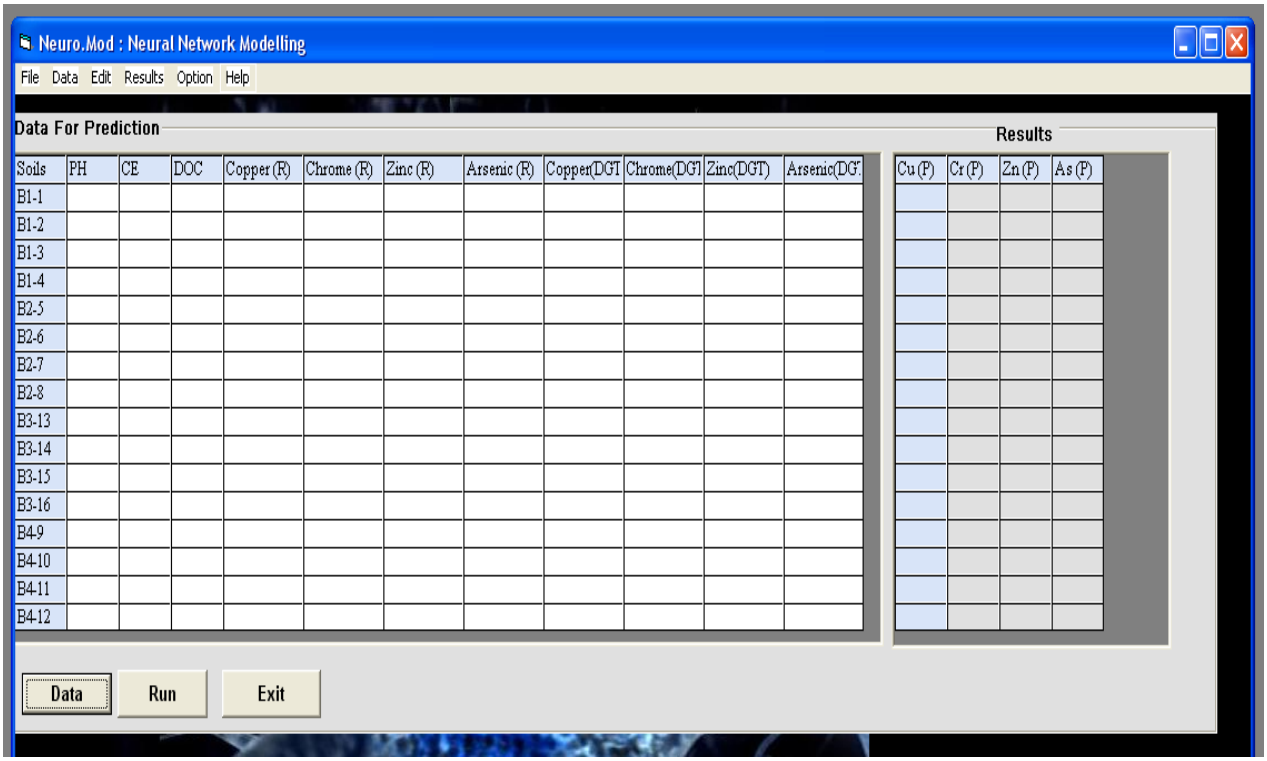


Figure 20. (Suite) Menu du lancement du calcul.

3. Affichage des résultats sous forme de courbes /graphiques de type Excel (Fig. 21).

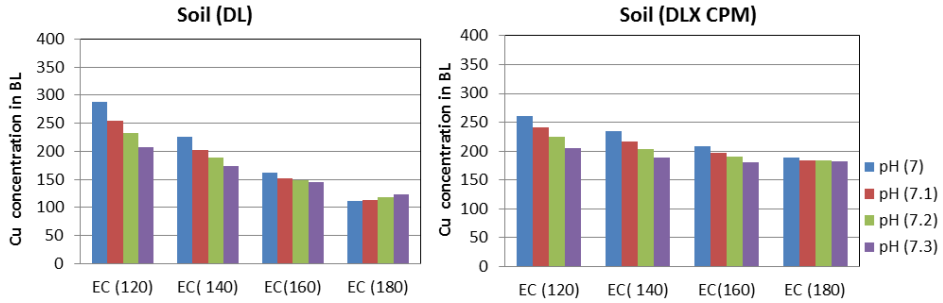


Figure 21. Menu d'affichage graphique.

4. Un menu de calcul inverse : on introduit une valeur cible de concentration d'un élément trace. Le calcul inverse prédit les valeurs des entrées permettant d'obtenir cette valeur cible (Fig. 22).

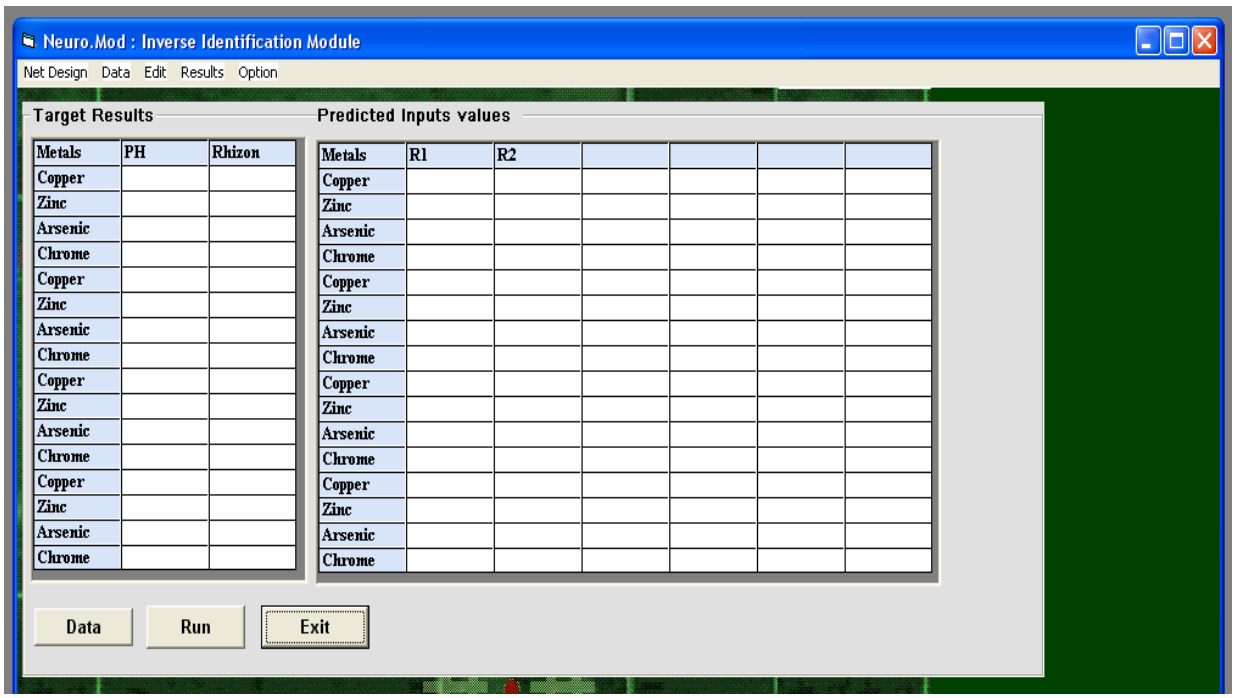


Figure 22. Menu de calcul inverse.

VB6 appelle le programme compilé en Fortran (fichier .exe) lorsqu'on clique sur le bouton Calcul (menu n°2).

Cette programmation hybride (VB6 pour l'interface graphique et Fortran pour le calcul ANN) permet une grande souplesse de programmation et une facilité d'utilisation par un non spécialiste de programmation.

III. Conclusion

Différentes approches de modélisation mathématique existent dans le domaine du sol. Chaque approche possède un domaine d'application spécifique et offre des avantages et des inconvénients qui sont résumés dans le tableau récapitulatif (Tab. 2) Il n'existe pas un type de modèle universelle capable de modéliser tous types de problèmes concernant les sols/sites contaminés.

Tableau 2. Récapitulatif des principales approches de modélisation, leurs avantages et inconvénients.

	Type du modèle	Auteurs	Avantages	Limitations
MODELES PHYSIQUES	**FIAM**	Morel, 1983	Ce modèle suppose: 1) les réactions de complexation de métaux et des ligands en solution sont sensiblement à l'équilibre; 2) la liaison des ions métalliques avec des sites de transport membranaire est proche de l'équilibre, et 3) la cinétique de transport transmembranaire est lente par rapport à la réaction de complexation de surface	Ce modèle a une utilité limitée pour la prédiction de la toxicité des métaux pour certains modes de fixation des métaux par le biote, y compris ceux des non-polaires espèces neutres-chargés (par exemple, HgCl2),sidérophore-complexes métalliques et des ligands de faible poids moléculaire (par exemple, le citrate) .
	MINEQL+	Schecher and McAvoy, 2001	Estimation de la distribution des métaux entre la phase liquide et la phase solide du sol	Ces modèles fonctionnent bien dans des conditions anoxiques, mais les estimations de liaison métalliques sont moins fiables dans des conditions anoxiques, où les concentrations de métaux sont le plus souvent contrôlées par la solubilité des sulfures métalliques. En outre, dans certaines circonstances, l'équilibre ne peut être atteint, en particulier lorsque le redox se déplace plus vite que la vitesse de réaction en métal ou lorsque la réaction est régie par des processus microbiens, comme c'est le cas avec la méthylation de Hg ou As
	MINTEQA2	Allison *et al.*, 1991		
	WHAM	Lofts and Tipping, 1998		
	PLANT X	Trapp et McFarlane, 1995	Plant X considère quatre compartiments différents des plantes : racine, tige, feuille, et fruit. Ce modèle sera donc utilisable pour modéliser le transfert vers tous les organes, y compris les fruits. La plupart des phénomènes de transfert sol-plante-air sont pris en compte, même si de nombreuses hypothèses simplificatrices sont émises.	Plant X ne prend pas en compte l'équilibre entre le sol et l'air, ces deux compartiments sont supposés être des sources inépuisables de polluants, avec une concentration constante dans ces deux compartiments environnementaux. Plant X ne considère pas le dépôt de particules et de gouttelettes sur les feuilles. Plant X ne prend pas en compte une éventuelle dissociation des composés organiques sous l'effet du pH.
MODELES CORRELATIVES	**ANN**	Haykin,1994, Heinzel,1997	Les réseaux de neurones présentent deux avantages fondamentaux : 1. Capacité à représenter n'importe quelle fonction, linéaire ou pas, simple ou complexe. Les réseaux de neurones sont ce qu'on appelle en mathématiques des approximateurs universels. (théorème de Kolmogorov, 1957) 2. Faculté d'apprentissage à partir d'exemples représentatifs, par retro propagation des erreurs	Le réseau de neurones fournit des réponses, mais pas des explications. En effet, l'apprentissage analyse les corrélations (associations intuitives) et non pas les causes et les conséquences (explications).
	MLR et RSM	Tiktak *et al.*, 1998, Hambli *et al.*, 2005	La MLR peut aider à trouver et comprendre la relation entre les facteurs X d'entrée et les réponses Y	La relation entre les entrées (Facteurs X) et les sorties (réponses Y) doivent être connues à l'avance afin que le modèle puisse les prédire

Typiquement, on cherche à prédire ou estimer des sorties représentants des réponses présentant un intérêt vis-à-vis de la problématique posée. Le problème réside dans la sélection et/ou le développement (création) d'un modèle représentatif et fiable. Cette phase nécessite généralement un savoir-faire mathématique et des

connaissances évoluées pas toujours disponibles. Proposer une approche de prédiction rapide, simple et fiable présente un très important avantage et atout pour modéliser les problèmes de sols. L'application des ANN répond à ces exigences d'autant plus que la méthode permet de modéliser des entrées et sorties de nature alphanumériques (attributs). Par exemple pour modéliser l'effet d'un amendement ou la nature d'un sol ; ceci reste problématique pour une modèle de type MLR ou RSM à cause de leur incapacité de représenter des attributs (sols1, sol2, amendement A, amendement B, etc…).

Notons que la présente revue est loin d'être exhaustive. D'autres types de modélisation ont été développés tels les modèles géostatiques, modèles basés sur les automates cellulaires (Cannataro *et al.*, 1995), systèmes experts (Chen *et al.*, 2003 ; Oprea, 2005), les modèles statistiques (Krahe *et al.*, 2006). L'objectif principal du chapitre était de donner une présentation générale des principales approches de modélisation mathématique dans le domaine des sols contaminés. L'accent a été mis sur la description de la modélisation par réseaux de neurones artificiels, approche utilisée dans le cadre de la thèse.

Les articles suivant ce chapitre (Chapitre 3.1, chapitre 3.2 et chapitre 3.3) représentent un apport innovant et important dans le cadre de cette thèse. En effet, trois modèles ANN ont été développées pour modéliser quelques problèmes de contamination de sols répondant à des problématiques scientifiques et applicatives différentes :

(i) prédiction sorties en fonction d'entrées
(ii) prédiction inverse : quelles sont les valeurs d'entrées assurant une sortie cible ?
(iii) modélisation stochastique d'un problème de type entrées-sorties tenant compte de la variabilité statistique (loi normale) des entrées.

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Chapitre.1.4

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2éme Partie

Résultats par site

Au cours de cette partie, nous allons présenter successivement les résultats obtenus sur les deux sites présentés dans l'introduction : le premier site est un sol naturel contaminé par une forte concentration d'éléments traces métalliques et spécialement en Cu. Sur ce site deux techniques de phytoremédiation ont été appliquées (phytosyabilation/phytoextraction) pour tester leurs capacités soit à fixer ou extraire ces éléments traces des sols. Le deuxième site est un technosol (anthroposol) contaminé également par une forte concentration en éléments traces et spécialement par le molybdène et le chrome. Sur ce site on a étudié la capacité de la phytostabilisation assistée à diminuer la mobilité de ces éléments potentiellement toxiques vers la nappe phréatique. Ensuite on a essayé d'étudier l'effet des substances humiques sur la mobilité et la disponibilité de ces éléments dans ce sol.

Nos résultats sont présentés sous forme d'articles (acceptés ou en révision ou soumis ou en préparation). Les résultats sont exposés en fonction des sites d'études et des techniques appliquées sur ces sites contaminés en éléments traces. Les chapitres 2.1 et 2.2 présentent les résultats obtenus sur l'application des technologies de phytostabilisation/phytoextraction sur un sol naturel contaminé en Cu. Les chapitres Chapitre 2.3 et 2.4 présentent les résultats obtenus par l'application des technologies de phytostabilisation sur un techno-sol fortement contaminé en ET et les résultats obtenus sur l'effet des substances humiques sur la mobilité des ET.

Cette partie est subdivisée en quatre chapitres.

Chapitre 2.1: Mobilité et phytodisponibilité du Cu, Cr, Zn et As dans un sol contaminé (site de préservation du bois) après quatre années d'application de la phytostabilisation assistée avec des amendements organiques et minéraux.

Chapitre 2.2: Phytoextraction assistée des éléments traces potentiellement toxiques d'un sol contaminé au cuivre avec une rotation de cultures de tabac et de tournesol sur deux années.

Chapitre 2.3: Phytostabilisation assistée d'un techno-sol contaminé par une forte concentration d'éléments traces, par des amendements organiques frais et matures

Chapitre 2.4: Effet des amendements organiques sur la mobilité des éléments traces dans un techno-sol contaminé phytoremédié: rôle des substances humiques.

Chapitre 2.1.

Mobilité et phytodisponibilité du Cu, Cr, Zn et As dans un sol contaminé (site de préservation du bois) après quatre années d'application de la phytostabilisation assistée avec des amendements organiques et minéraux

2.1.1. Résumé de la publication

Dans cet article, nous avons évalué la capacité de la phytostabilisation à l'aide de peupliers mycorhizés et de saules assistée par des amendements organiques tels que le compost (OM, 5% p/p) et inorganiques tel que le calcaire dolomitique (DL, 0,2% p/p), seuls ou en association (OMDL) à diminuer la mobilité, la disponibilité, l'intensité d'exposition du sol et la phytodisponibilité du Cu, Zn, Cr et As dans un site de préservation du bois situé dans le sud-ouest de la France fortement contaminés en sulfate de Cu et en sels e Cu (CCA).

Pour réaliser l'objectif de notre travail, 16 échantillons de terre (4 sols pour 4 amendements) ont été collectés sur les 16 parcelles du site (10 cm de profondeurs, séché à l'air et tamisé à 4mm) et mis en pot au laboratoire (1kg). Les concentrations dissoutes totales en éléments potentiellement toxiques (PTTE) ont été déterminées dans l'eau interstitielle du sol (SPW) collectée par des échantillonneurs Rhizon après avoir augmenté l'humidité du sol jusqu'à 80% de la capacité au champ. L'intensité de l'exposition du sol a été évaluée par des sondes Chelex100-DGT (peepers-gels), qui ont été insérées manuellement pendant 24 heures directement dans le sol humide (80%) de chaque pot. La phytodisponibilité résiduelle des PTTE après les différents traitements a été caractérisée par la croissance de haricots nains sur les mêmes échantillons de sols en pot: OM, DL, OMDL et CTRL. Dans ce test de germination on a mesuré les concentrations foliaires et la minéralomasse des PTTE accumulé dans les feuilles d'haricots. Toutes les analyses élémentaires- eau interstitielle, concentrations foliaires- concentrations disponible (DGT) ont été obtenues par ICP-MS (ICP-MS (Element 2, Thermofischer).

Nos résultats ont montré que la concentration de Cu dans les SPW a augmentée dans les sols amendés avec la OM par rapport aux autres sols amendés et le sol de contrôle (un sol de jardin de la même terrace alluviale), mais elle est restée inchangée dans les sols DL et OMDL. Le Cu disponible déterminé par DGT utilisée pour évaluer l'intensité de l'exposition du sol a diminué dans tous les sols contaminés amendés par rapport au sol contaminé sans amendement. Cette concentration du Cu disponible déterminé par DGT à été corrélée avec la concentration foliaire de Cu. Les concentrations de Zn dans les SPW des sols amendés ont été réduites et notamment dans le sol traité avec le DL, tandis que ces amendements n'ont pas eu un effet significatif sur les concentrations disponibles du Zn dans les SPW. Tous les amendements ont augmenté la concentration d'As dans les SPW du sol contaminé par rapport au sol sans amendement. Basée sur des données DGT, la disponibilité du Cu a été réduite à la fois pour les sols OM et les sols OMDL, tandis que DL a été le plus efficace pour

diminuer la disponibilité du Zn. Les sols amendés avec OM et DL ont augmenté la concentration du Cr dans les SPW des sols amendés par rapport au sol sans amendement.

Nos résultats ont montré également que les amendements OM et DL, seuls ou en combinaison (OMDL), ont été efficaces pour diminuer les concentrations foliaires de Cu, Cr, Zn et As dans les feuilles de haricots par rapport au sol sans amendement, les valeurs les plus basses étant obtenues pour les haricots cultivés dans le sol phytostabilisé avec l'amendement OM. Les sols amendés avec OM et DL ont la capacité à diminuer les minéralo-masses de Cu, Cr et As calculées dans les feuilles primaires de haricots, mais ça n'a pas été le cas pour le Zn.

Finalement nous pouvons dire que la phytostabilisation assistée par des amendements a la capacité à réduire les concentrations de quelques PTTE mesurés dans les SPW notamment le Cu, mais pas tous. Par contre cette option de phytomanagement a été efficace pour réduire les concentrations disponibles des PTTE notamment le Cu et pour diminuer les concentrations foliaires et les minéralomasse des PTTE.

2.1.2. Article 1 (en préparation)

Mobility and phytoavailability of Cu, Cr, Zn, and As in a contaminated soil at a wood preservation site after four years of aided phytostabilization

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Keywords: Soil contamination, Copper, Amendment, phytoavailablity, phytostabilisation

Abstract: Copper-contaminated soils were phytomanaged by aided phytostabilisation in 16 field plots at a wood preservation site. The mobility and bioavailability of four potentially toxic trace elements (PTTE), i.e. Cu, Zn, Cr, and As, were investigated in these soils four years after the incorporation of compost (OM, 5% w/w) and dolomite limestone (DL, 0.2% w/w), singly and in combination (OMDL), and the transplantation of mycorrhizal poplar and willows. Topsoil samples were collected in all plots and potted. Total PTTE concentrations were determined in soil pore water (SPW) collected by Rhizon soil moisture samplers. Soil exposure intensity was assessed by Chelex100-DGT (diffusive gradient in thin films) probes. The PTTE phytoavailability was characterized by growing dwarf beans on potted soils and analyzing their foliar PTTE concentrations. OM and DL, singly and in combination (OMDL), were effective to decrease foliar Cu, Cr, Zn, and As concentrations of beans, the lowest values being numerically for the OM plants. The soil treatments did not reduce the Cu and Zn mineral masses of bean primary leaves, but those of Cr and As decreased for the OM and DL plants. The Cu concentration in SPW was increased in the OM soil and remained unchanged in the DL and OMDL soils. The available Cu measured by DGT used to assess the soil exposure intensity correlated with the foliar Cu concentration. The Zn concentrations in SPW was reduced in the DL soil. All amendments increased As in SPW. Based on DGT data, Cu availability was reduced in both OM and OMDL soils, while DL was the most effective to decrease soil Zn availability.

1. Introduction

Soils contaminated by PTTE have serious consequences for terrestrial ecosystems, agricultural production and human health (Adriano, 2001; Alloway, 1995). Such soil contamination is often accompanied by a loss of biodiversity and land cover, notably at sites where Cu-based salts have been either long-term produced or used (Freitas et al., 2004; Bes et al., 2010). The fate of PTTE in soils is influenced by physical and chemical reactions between the solid components of soil and the liquid phase (Morel et al., 2006). Soil factors such as pH, soil organic matter (SOM), texture, redox potential, and temperature (Alloway, 1995) and biological processes controlled by soil micro-organisms and plants are key-players in the root zone for the PTTE mobility and bioavailability (Chaignon et al., 2002; 2003). Roots can indeed modify the PTTE mobility by changing soil pH, electrochemical potentials through element

sorption in apoplast and functioning of membrane transporters, and their rhizodeposition including soluble root exudates and mucilages (Hinsinger, 1998, 2001a, b; Lombi et al., 2001; Chaignon et al., 2002).

Conventional soil reclamation technologies such as 'dig and dump', soil washing, and sieving are effective but destructive, thus not sustainable in terms of consumption of raw materials and waste production, and costly for large contaminated sites (Basta et al., 2004; Raicevic et al., 2005; Dermont et al., 2008). Less invasive, low-cost phytotechnologies such as phytostabilization, singly and in combination with in situ stabilization (i.e. aided phytostabilisation), are potential options to restore the physical, chemical, and biological properties of PTTE-contaminated soils (Mench et al., 2000; Bolan et al., 2003; Pérez de Mora et al., 2005; Raicevic et al., 2005; Kumpiene et al., 2006; 2008). Several mineral and organic amendments such as lime, coal fly ashes, phosphates, red muds, compost, biosolids, iron grit and Fe/Mn/Al oxides can improve phytostabilization and production of plant-based feedstock through decrease in PTTE bioavailability (Lombi et al., 2002; Bolan et al., 2003; Brown et al., 2004; Geebelen et al., 2003; Basta et al., 2004; Kumpiene et al. 2008; Mench et al., 2010). Case studies assessing the PTTE mobility and bioavailability in contaminated soils long-term managed by (aided) phytostabilisation are needed to better define the pros and cons of such management options (Mench et al., 2010). Therefore, this work aimed at assessing the mobility, soil exposure intensity, and phytoavailability of Cu, Zn, Cr, and As in top soils (0-25 cm) of 16 field plots at a wood preservation site in Southwest France, four years after their implementation for testing four options of (aided) phytostabilization. These options were either to incorporate OM and DL, singly and in combination (OMDL), or not into the contaminated soil prior the transplantation of mycorrhizal poplar and willows. Total dissolved concentrations of PTTE were quantified in SPW collected by Rhizon soil moisture samplers to assess PTTE mobility. Soil exposure intensity of Cu and Zn was determined by DGT probes. The PTTE phytoavailability was characterized by cultivating dwarf beans and analyzing their primary leaves. The efficiency of the four phytoremediation options to reduce the phytoavailability of Cu, Cr, Zn, and As was compared.

2. Material and Methods

2.1. Site, soil sampling and soil characterization

The wood preservation site (6 ha are partially active and 4 ha with historical activities were used for an allotment) is located in the Gironde County (44°43'N; 0°30'O), Southwest France. It has been used for over a century to preserve and store timbers, posts and utility poles and various Cu-based salts were successively utilized (Mench and Bes, 2009). Plant communities and soil characteristics were previously assessed, and Cu is the main contaminant in topsoils (i.e. 65 to 2600 mg kg^{-1}, Mench and Bes, 2009; Bes et al., 2010; 2012). Soil Cu contamination mainly results from washings of treated timbers. Plant communities in the zone of the field trial included *Agrostis capillaris, Elytrigia repens, Rumex acetosella, Portulaca oleracea, Hypericum perforatum, Hypochaeris radicata, Euphorbia chamaescyce, Echium vulgare, Agrostis stolonifera, Lotus corniculatus, Cerastium glomeratum,* and *Populus nigra* (Fig. 1a) (Bes et al., 2010). The geological structure of the site consists of two layers. The first one is a mixture of brown sand and gravels, from the medium Pleistocen (Riss); with a depth in the range 2.5- 4.5 m whereas the second layer is composed of marls and decalcification clays related to eroded Stampien materials (Mench et al., 2008). Fifteen sub-sites were previously defined (labeled from A to E and P1 to P10) depending on total topsoil Cu concentration (Fig. 1b) (Mench and Bes, 2009; Bes et al., 2010). Long-term phytostabilization experiments are established at the P3 and P7 sub-sites. The field trial (150 m²) established in 2006 at the site P1-3, formerly used for stacking treated wood and utility poles (Fig. 1b, Bes, 2008; Lagomarsino et al., 2011), is cultivated as a short rotation coppice including mycorrhizal poplar (*P. nigra* L.) and willows (*Salix caprea* and *S. viminalis)* (Bes, 2008). It consists in 16 plots (1 m x 3 m) that have received one of the following four initial treatments randomly replicated in four blocks, i.e. untreated (UNT), dolomitic limestone (DL, 0.2% by air dried soil, w/w,

NF U 44 001, 30% CaO and 20% MgO combined with carbonates, 80% < 0.16 cm, Prodical Carmeuse, Orthez, France), compost (OM, 5% w/w), and DL combined with OM (OMDL) (Fig.1c). Compost derived from composting (9-12 months) poultry manure and pine bark chips (ORISOL, Cestas, France, Bes and Mench, 2008). Soil amendments were carefully mixed in the topsoil (0-0.30 m) with a stainless spade. Topsoils (0-25 cm, alluvial origin, Fluviosol) were sampled in April 2010 (average sample made of three sub-samples of 1 kg) with a stainless spade in the 16 plots. Main characteristics of topsoil's at the site P3 are presented in Table 1. Their texture is sandy. Organic matter content is low as well as cation exchange capacity (CEC). Total soil concentrations were in the common range of French sandy soil for Cr, As, and Zn but total soil Cu was in excess for these coarse sandy soils *i.e.* 35 mg Cu kg^{-1} (Tab. 1, Baize et al., 1997; 2002).

Figure 1. (a) location of the studied site, the arrow indicating the site P3, (b) implementation of the plots of the field trial (2006) carried out with soil amendments and mycorrhizal trees since 2006 at the Biogeco phytoremediation platform (adapted from Bes et al.2010) and (c) Photo of the field plots (P3 sub-site) in April 2010, 4 replicated blocks, each block treated with 4 types of amendements (OM, DL, OMDL and UNT)

Table 1. Main characteristics of the P3 and control soils (0-0.25 m soil layer)

Parameters	Site P3	Control soil	Background values in French sandy soils[a]
Sand %	83.5 ± 1.1	66.5	
Silt %	11.5 ± 0.9	15.5	
Clay %	3.8 ± 1.2	18.0	
C/N	17.2	13.8	
SOM (g. kg^{-1})	15.9	69.9	
CEC (cmol+/kg)	3.49	16.1	
organic C (g. kg^{-1})	9.19	40.4	
total N (g. kg^{-1})	0.534	2.94	
pH	7.0 ± 0.23	7.01	
As (mg.kg^{-1})	9.8	3.6	1.0-25[b]
Co (mg.kg^{-1})	2	2.62	1.4-6.8
Cu (mg.kg^{-1})	674 ± 126	21.5	3.2-4.8
Cr (mg.kg^{-1})	23	17.9	14.1-40.2
Mn (mg.kg^{-1})	181	189	72-376
Ni (mg.kg^{-1})	5	7.46	4.2-14.5
Zn (mg.kg^{-1})	46	50.9	17-48

[a] median and high vibrissae values except for As (Baize., 1997; Baize and Tercé, 2002):. [b] common As mean values for all French soil types (Baize and Tercé, 2002).

2.2. Germination tests

For each of the 16 plots, 1 kg of soil was potted after sieving (2 mm). Similarly two samples of an uncontaminated control soil (CTRL) from a kitchen garden (0-0.25 m Gradignan, France) of the same alluvial terrace were taken and potted (Tab 1). Four seeds of dwarf beans (*Phaseolus vulgaris*) were sown in all pots and cultivated for 18 days in controlled conditions (16 h light/8 h darkness regime,

25°C/21°C). The soil moisture was maintained at around 50% of the field water capacity with additions of deionized water after weighing; then the soil moisture was raised to 80% at the beginning of seed germination. At harvest, the dry weight (DW) of bean primary leaves (BLDW) was determined after drying at 70°C. Aliquots of primary leaves (BL) were weighed (35-150 mg) directly into Savillex Polytetrafluoroethylene PTFE 50 mL vessels, 2 mL H_2O and 2 mL of 14M HNO_3 were added and heated open at 65°C for 2 hours. Then the caps were closed and the containers were left overnight at 65°C (12-14h). Thereafter, they were opened, 0.5 mL of H_2O_2 (30%) was added to each sample and left at 75°C open for 3 hours. Then 1.5 ± 0.5 mL of *fluorhydric acid* (HF, 48%) was added to each sample, caps closed and left at 100°C overnight. Containers were opened and kept at 120°C for 4-5 hours evaporating to dryness, taken off heat, 1mL HNO_3 + 5mL H_2O + 0.1 mL H_2O_2 were added to each, gently warmed up (65°C) and after cooling down made up to 50 mL with distilled water**.** Mineral composition of the digests was analyzed by ICP-MS (Varian 810-MS) and elemental concentrations of the BL were determined for Cu, Cr, As, and Zn. All foliar element concentrations are expressed in mg kg^{-1} DW. The mineral mass of each PTTE in BL was computed based on their elemental concentrations and the BLDW.

2.3. Characterization of soil pore water

SPW were extracted from each pot by Rhizon soil moisture samplers (SMS, model MOM, Rhizosphere Research Products, Wageningen, The Netherlands) with a nominal porosity of 0.15 µm after harvesting the dwarf beans. The capped end was inserted into each potted soil during filling. A syringe needle was connected to the female lock and inserted into a 10 mL glass vacuum tube, for extracting the SPW by vacuum (Cattani, 2006). Before putting the samplers in the soils, the SMS devices were previously cleaned with 5% HNO_3 and then washed twice with deionised water.

Three SMS devices were placed at 45° in the soils. The soil moisture was maintained at 80% for 15 days. Each device was then let under vacuum for 24 hours for collecting the soil pore water (SPW, ~ 30 mL) from all pots (18 soil samples, three SMS/pot). The SPW were stored at 4°C. An aliquot (3 mL) of each SPW was acidified with 0.1 M HNO_3 for measuring the concentrations of Cu, Zn, Cr, and As in the SPW samples by HR-ICP-MS (Element 2, Thermofischer). In all SPW samples, dissolved organic carbon (DOC) was determined by a carbon analyzer (Shimadzu© TOC 5000A), and concentrations of major cations (Na^+, K^+ and Ca^{2+}) and anions (NO_3^-, SO_4^{2-}, and Cl^-) were analyzed by ionic chromatography (Dionex ICS-2000, Sunnyvale, CA using the columns CS16A for measuring cations and AS17 for anions).

2.4. DGT measurements

For measuring available metals in the soils and characterizing the soil exposure, standard cylindrical Chelex 100-DGT units with an active surface area of 3.14 cm^2 were manually inserted for 24 h directly into the humid topsoil (80%) of each pot. The DGT probes consist of three layers: the first one is a 0.45µm filter; the second layer is a diffusion layer which consists of a polyacrylamide gel layer and the third one a polyacrylamide gel layer that incorporates a Chelex-100 resin that binds strongly the labile trace metal species (Davison et al., 2000; Ernstberger et al., 2002).

DGT continuously accumulates metals on the resin gel during deployment. The total mass of each metal (*M*) accumulated per unit area over the deployment time (*T*) is given by integrating the flux over the deployment time (eq. 1):

$$M = \sum_{t=0}^{T} F(T)dt \qquad \text{(eq. 1)}$$

The total mass of each metal *(M)* is determined analytically through the area exposed to the solution *(A)* by measurement of the eluent concentration (*C*e) after elution of the resin gel (volume, *V*gel) with 1 M HNO_3 (volume, *V*HNO3),

$$M = C_e (V_{HNO3} + V_{gel})/fe \qquad \text{(eq. 2)}$$

With f=0.8 for Cu, Cr and Zn.

The strong binding of metals in the resin gel leads to the creation of a linear concentration gradient in the diffusive gel. This gradient depends on several factors such as the interfacial concentration of labile trace metal species, C_i and the thickness of the diffusion layer, Δg (cm), All these factors determines the flux, $F(t)$, of metal from the soil to the resin-gel according to Flick's first law (eq. 3):

$$F(t) = \emptyset \mathrm{d} D \mathrm{d} \frac{Ci(t)}{\Delta g} \qquad \text{(eq. 3)}$$

where $\emptyset \mathrm{d}$ is the porosity of the diffusion gel and $D\mathrm{d}$ (cm^{-2} s^{-1}) is the diffusion coefficient of the labile trace metal species in the diffusion layer. The diffusion coefficients were taken from Zhang et al. (2001).

The averaged interfacial concentration, C_{DGT}, or available concentration can be calculated from M (eq. 4)

$$C\mathrm{DGT} = \frac{M \Delta g}{\emptyset \mathrm{d} D \mathrm{d}} \qquad \text{(eq. 4)}$$

For each metal, the division of the available concentration (C_{DGT})on the total concentration measured in the SPW, C_{SPW}, give the ratio, R, which indicates the extent of the depletion of soil pore water concentrations at the DGT interface (eq. 5)

$$R = C_{DGT}/C_{SPW} \qquad \text{(eq. 5)}$$

For each metal, the mass accumulated in the resin-gel layer was determined after extraction of the resin gel by 1 mL of HNO_3 5% for 24h. This solution was further diluted 10 times and analyzed by HR-ICP-MS (Element 2, Thermo Fischer) for determining metal concentrations (Cu, Cr and Zn).

2.5. Statistical analysis

Analysis of variance (ANOVA), Tukey Post Hoc test (Statistica) and Pearson correlation coefficients (linear regression) (significance level, $p<0.05$) were performed on the total SPW concentrations, DGT concentrations, R ratios, foliar element concentrations, foliar mineral masses of elements and leaf DW yields to evaluate the treatment influence. All analytical determinations were performed in four replicates. Differences were considered statistically significant at $p < 0.05$. All statistical analyses were performed using the statistical software Statistica (version 6).

3. Results and discussion

3.1. Soils and soil pore waters

Table 2 shows the physico-chemical parameters of soils (i.e. pH, EC and TOC), and of SPW (i.e. DOC and major cation and anion concentrations) depending on soil treatments, i.e. phytostabilisation (Unt) and aided phytostabilisation (OM, DL, and OMDL). Aided phytostabilisation based on amendment incorporation into the soil followed by tree transplantation increased slightly the soil pH from 7.16 up to 7.45 in the amended soils compared with the unamended one, mean soil pH values following the decreasing order: DL > OMDL > OM > UNT > CTRL. However, differences in the soil pH and TOC (0.53-1.68%), and in DOC of SPW (29-63 mg C L^{-1}) were only significant between the contaminated soils (UNT, OM, DL, and OMDL) and the uncontaminated control soil (CTRL) ($P=0.0019$, $P= 1.45E^{-6}$, $P= 0.0013$). They were insignificant between the amended and unamended contaminated soils. The soil EC values varied in the 157-192 µS cm^{-1} range but did not differ between all investigated soils.

Four years after, amendments added to the contaminated soils had little influence on cation concentrations in the SPW (Tab. 2). The SPW Mg^{2+} concentrations varied from 10.9 mg (Unt) to 41.3 mg L^{-1} (DL) and was significantly higher for the DL soil compared to the UNT soil ($P=0.014$), The SPW Na^{+}, K^{+}, and Ca^{2+} concentrations were respectively in the 8-38 mg. L^{-1} Na, 16-49 mg. L^{-1} K, and 87-272 mg. L^{-1} Ca. L^{-1} ranges, but differences between soils were insignificant. For the anion concentrations in the SPW, Cl^{-}, NO_3^{-}, and SO_4^{2-} varied respectively between 9.6-42.9 mg. L^{-1}, 300-914 mg. L^{-1}, and 13.9-42.7 mg. L^{-1}, without significant differences across the soil series (Tab. 2).

Metal concentrations in SPW generally mirror root exposure to metals (Sauvé et al. 1997; Sauvé 2003; Tandy et al. 2006; Forsberg et al. 2009). Soil pore waters collected from the potted

contaminated and uncontaminated soils showed significant differences in total metal concentrations (Fig. 2). On this soil series, total metal concentrations in the SPW were generally in decreasing order: Cu > Zn > As > Cr (mean values in µg L^{-1} on the soil series: Cu 676, Zn 22, As 3.0, and Cr 0.68). This ranking reflected total metal concentrations in the soils, except that total soil Cr was higher than total soil As (Tab. 1).

Table 2. Physico-chemical characteristics of the soils and soil pore water

Soils	pH	EC (µS. cm^{-1})	TOC %	DOC (mg C.L^{-1})	Cations (mg. L^{-1})				Anions (mg. L^{-1})		
					Na^{+} ± SD	Mg^{+2} ± SD	K^{+} ± SD	Ca^{+2} ± SD	Cl^{-} ± SD	NO_3^{-} ± SD	SO_4^{-2} ± SD
UNT	7.16±0.12 a**	161±64 NS	0.64±0.02 a***	29.4±8.8 a**	21.0±6.6 NS	10.9±6.3 a*	21.9±19.6 NS	156.4±56.5 NS	16.9±2.1 NS	494.9±206.7 NS	19.6±4.2 NS
OM	7.27±0.38 a**	168±51 NS	1.68±0.33 a***	38.0±7.1 a**	38.7±35.3 NS	22.9±14.0 ab*	41.4±26.6 NS	166.8±114.2 NS	42.9±45.7 NS	548.0±355.0 NS	39.3±28.4 NS
DL	7.45±0.15 a**	157±16 NS	0.53±0.05 a***	39.4±6.5 a**	28.2±11.9 NS	41.3±18.9 b*	24.7±18.4 NS	272.6±147.8 NS	26.7±17.8 NS	914.4±527.2 NS	42.7±28.0 NS
OMDL	7.32±0.11 a**	192±39 NS	0.72±0.09 a***	40.9±4.7 a**	19.5±5.7 NS	21.8±9.4 ab*	16.4±3.8 NS	87.5±41.7 NS	18.3±7.2 NS	300.8±165.8 NS	18.4±7.7 NS
CTRL	6.45±0.07 b**	169±22 NS	0.62±0.30 b***	63.06±1.7 b**	8.6±8.5 NS	23.2±10.7 ab*	49.7±7.9 NS	113.7±60.8 NS	9.6±12.8 NS	*ND*	13.9±9.8 NS

0.01<(*)P <0.05, 0.01>(**) p >0.001, (***) p< 0.0001, (NS) insignificant difference; ND: not determined. Unt : untreated, OM : Compost, DL : Dolomitic limestone, OMDL : compost and dolomitic limestone, CTRL : uncontaminated control soil. EC: electrical conductivity, TOC: total organic carbon; DOC: dissolved organic carbon

3.1.1. Soil Cu exposure

The addition of OM and OMDL numerically increased 2 fold and 1.3 fold respectively the total dissolved Cu concentration in the SPW (Cu_{spw}) whereas the DL treatment slightly decreased it (0.8 fold) compared to the UNT soil, however only the OM soil differed from the UNT soil (Fig. 2a). The Cu_{spw} value of the CRTL soil was 58%, 79%, 52% and 67% lower than those of the UNT, OM, DL and OMDL soils, respectively, i.e. 2-5 fold less than for these contaminated soils. Compost incorporation into the contaminated soil significantly increased Cu_{spw} compared to other amendments (P=9.0 E^{-19}) and the uncontaminated soil (CRTL) (P=1.12 E^{-10}) (Fig. 2a). All amendments significantly decreased the available Cu concentration (Cu_{DGT}) in the contaminated soils by roughly a factor 2 (P= 1.38 E^{-14}) (Fig. 2e). Cu_{DGT} peaked in the UNT soil and was the lowest in the CTRL soil (P = 5.4 E^{-16}). In contrast with Cu_{spw} (Fig. 2a), Cu_{DGT} was lower in the OM and OMDL soils than in the DL soil (Fig. 2e). The Cu_{spw} values in the OM and OMDL soils were respectively 7 fold and 5 fold higher than the Cu_{DGT} values, i.e. (in µg Cu L^{-1}) OM: 1065 and 157 respectively; OMDL: 665 and 147, respectively) (Fig.2. a and e). Similar comparison showed a 2-fold factor for the UNT soil (519 µg and 280 µg Cu L^{-1}) and the DL soil (456 µg and 197 µg Cu L^{1}).

On the whole soil series, the Cu_{spw} values were not correlated to the Cu_{DGT} ones (r= -0.16 the correlation in the contaminated and the control soil, r=-0.57 the correlation calculated in the contaminated soil without the control one), but highly positively correlated with the soil TOC values (r = 0.86 "contaminated soil with control one") (Tab. 3). The correlation between [Cu_{spw}] and TOC was even stronger when only the Cu-contaminated soils are considered (r = 0.93) (Tab. 4). The Cu_{spw} did not significantly correlate to soil pH (r=0.35 "in the presence of control soil, r=-11 only in contaminated soils, Tab. 3), The correlation was significant and negative between Cu_{DGT} and the DOC measured in the SPW (r=-0.68) (Tab. 3,4).

Table 5 presents the R ratio (R_{Cu} = Cu_{DGT}/Cu_{spw}) values for the whole soil series. The R_{Cu} values of all amended soils were lower than that for the UNT soil. The soil amendments reduced R_{Cu} and led to the increasing order: OM < OMDL < DL < UNT, this reduction reaching respectively 73% (OM), 59% (OMDL), and 20% (DL) compared to the UNT soil. The R_{Cu} of the control soil was 35% lower than that of the UNT soil.

The Cu phytotoxicity in soils depends mainly on its solubility and chemical speciation, which are influenced by its sorption onto mineral and SOM (Garrido et al., 2005). Here total dissolved Cu concentration in SPW increased significantly in the OM-amended soils and slightly in the OMDLsoils (Fig. 2a). The DOM (dissolved organic matter) may mobilize soil Cu and bound it in the SPW (Beesley and Dickinson, 2011). However, four years after adding OM and OMDL, the DOC in SPW as well as soil pH were rather similar in both compost-amended soils and the DL soil (Tab. 2). One week after compost incorporation into the soil, Cu mobility can be reduced by 71% (Ruttens et al., 2006). The SOM and DOM in the SPW react with Cu, and their complexes modify Cu solubility, chemical species, and re-supply from soil bearing phases (Ashworth and Alloway 2007; Temminghoff et al. 1997). Fulvic acid bound Cu in the SPW, increases its mobility from the solid phase to the liquid phase of soils, notably in compost-amended ones (Hsu and Lo, 2000). Soluble Cu may increase in the SPW in the presence of DOM whereas high molecular mass organic compounds can sorb Cu (McBride and Martinez, 2000; Docekal et al., 2005). After one year, Cu bound to organic matter increased 3-fold in compost-amended soils at this wood preservation site (Lagomarsino et al. 2011). The Cu–SOM complexes, particularly with non-soluble, high molecular mass organic acids can decrease Cu phytoavailability (Chirenje and Ma, 1999; Balasoiu et al., 2001; Bolan and Duraisamy, 2003). Regulation of Cu mobility from soil to plants by SOM in relation to the ligand functional groups was suggested by Hsu and Lo (2000) and Thakali et al. (2006). In calcareous soils OM may reduce the retention of Cu by $CaCO_3$, and increase its solubility in the soil (Shuman. 1986; Saha et al.1991) as in the OMDL soil (Fig. 2a). The insignificant, small reduction in Cu_{spw} (0.8 fold) in the DL soil can be explained by the slight increase in soil pH (Sauve´ et al., 1997). The DL addition may limit Cu_{spw} by Cu precipitation and complexation and increasing the Cu sorption on solid bearing phases such as organic matter, clays, Fe/Mn (hydr) oxides, carbonates and phosphates (Filius *et al.*, 1998; Kabata-Pendias and Pendias, 2000; Garrido et al., 2005 Kumpiene et al., 2006; Lagomarsino et al. 2011). The DL addition induced a similar trend when OM and OMDL soils were compared (Fig. 2a). Similarly, liming soils from pH 4.6 to 6.9 can decrease the water soluble and exchangeable Cu fraction from 11.7 to 4.6 mg kg^{-1} in sludged soils (Brallier et al., 1996).

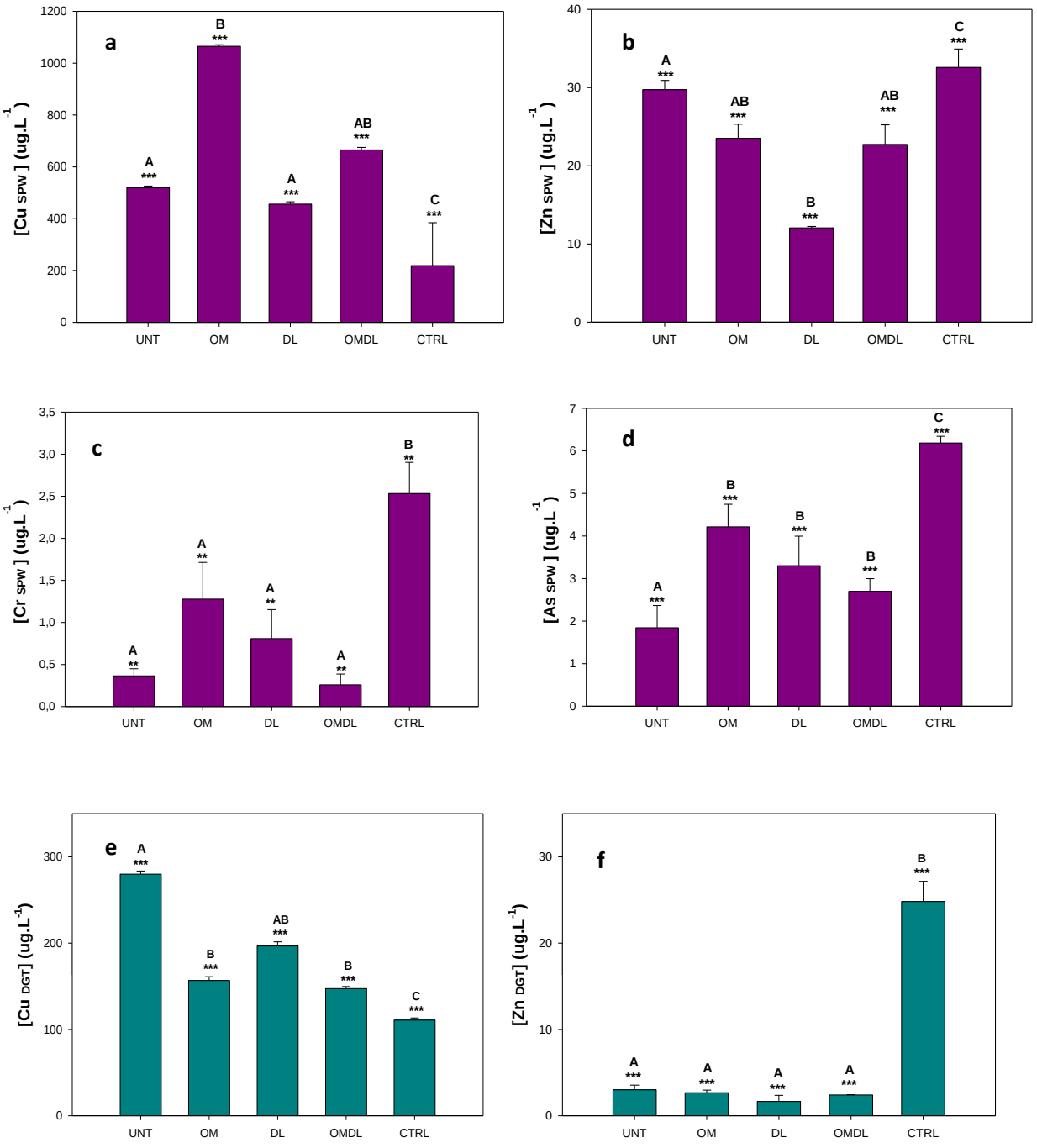

Figure 2. Concentrations of Cu, Zn, Cr, and As in the soil pore waters and intensity of Cu and Zn exposure in the soils determined by DGT. Values are mean ± standard deviation (n=4). Different letters on bar graphs indicate a significant difference ($p<0.05$)

Table 3: Pearson's correlation coefficients between the parameters of soils and soil pore waters of the contaminated and control soil.

	Amendement	pH	EC (µS. cm^{-1})	TOC (%)	DOC (mg.L^{-1})	[Cu tot]	[Cu spw]	[Cu $_{DGT}$]	R
Amendement	1.00								
pH	-0.56**	1.00							
EC (µS. cm^{-1})	0.10 ^{NS}	-0.17 ^{NS}	1.00						
TOC (%)	-0.26 ^{NS}	0.10 ^{NS}	0.10 ^{NS}	1.00					
DOC (mg.L^{-1})	0.83***	-0.47**	0.10 ^{NS}	-0.03 ^{NS}	1.00				
[Cu tot]	-0.65**	**0.61****	0.24 ^{NS}	0.14 ^{NS}	**-0.65****	1.00			
[Cu spw]	-0.49**	0.35 ^{NS}	0.09 ^{NS}	**0.86*****	-0.33 ^{NS}	0.46**	1.00		
[Cu $_{DGT}$]	-0.75***	0.23 ^{NS}	-0.18 ^{NS}	-0.29 ^{NS}	**-0.68****	0.30 ^{NS}	-0.16 ^{NS}	1.00	
R	-0.23 ^{NS}	-0.06 ^{NS}	-0.21 ^{NS}	**-0.69****	-0.26 ^{NS}	-0.12 ^{NS}	-0.71***	0.80***	1.00

Significance level: ^{NS} Not significant, * P<0.05, ** P<0.01, *** P<0.001.

TOC: total organic carbon, Cu_{DGT}: interfacial Cu concentration determined by DGT, Cu_{spw}: total Cu concentration in the soil pore water; DOC: dissolved organic carbon, EC: electrical conductivity, Cu tot: total soil Cu, Amendement: the four types of soil treatments (UNT, OM, DL, and OMDL).

Table 4: Pearson's correlation coefficients between soil and soil pore water parameters in the contaminated soil (without control).

	Amendement	pH	EC (µS. cm^{-1})	TOC (%)	DOC (mg.L^{-1})	[Cu tot]	[Cu spw]	[Cu $_{DGT}$]	R
Amendement	1.00								
pH	0.35 ^{NS}	1.00							
EC (µS. cm^{-1})	0.21 ^{NS}	-0.27 ^{NS}	1.00						
TOC (%)	-0.21 ^{NS}	-0.07 ^{NS}	0.11 ^{NS}	1.00					
DOC (mg.L^{-1})	0.54*	0.31 ^{NS}	0.18 ^{NS}	0.16 ^{NS}	1.00				
[Cu tot]	0.11 ^{NS}	0.62*	-0.08 ^{NS}	0.24 ^{NS}	0.46 ^{NS}	1.00			
[Cu spw]	-0.08 ^{NS}	-0.11 ^{NS}	0.09 ^{NS}	**0.93*****	0.14 ^{NS}	0.23 ^{NS}	1.00		
[Cu $_{DGT}$]	-0.76**	-0.25 ^{NS}	-0.21 ^{NS}	-0.43 ^{NS}	-0.55*	-0.32 ^{NS}	**-0.57***	1.00	
R	-0.47 ^{NS}	-0.09 ^{NS}	-0.21 ^{NS}	-0.70**	-0.41 ^{NS}	-0.26 ^{NS}	-0.84***	0.91***	1.00

Significance level: ^{NS} Not significant, * P<0.05, ** P<0.01, *** P<0.001.

Table 5: Capacity of the solid phase to resupply Cu, Zn, and Cr to the pore water: Ratio of the available concentration determined by DGT vs. total dissolved soil pore water concentration (C_{DGT}/ C_{spw})

Amendment	R(Cu)	R(Zn)
UNT	0.54 **a**±0.01	0.10 **a** ±0.02
OM	0.15 **b**±0.00	0.12 **a** ±0.01
DL	0.43 **ab**±0.02	0.16 **a** ±0.6
OMDL	0.22 **c**±0.01	0.15 **a**±0.03
CTRL	0.34 **abc**±0.01	0.76 **b** ±0.02

R > 0.95: high capacity of the solid phase to resupply the pore water ; R < 1 : the DGT device is supplied only by the diffusion of solutes through the pore water which becomes progressively depleted. Other R values : partial resupply from the solid phase, but insufficient to sustain fully pore water concentrations. Mean values in a column followed by the same letter did not differ at the 5% level

3.1.2. Soil Zn exposure

The Zn concentration in the SPW (Zn_{spw}) was reduced in the amended soils, i.e. OM: 21%, OMDL: 24% and DL: 59% ($P= 5.35E^{-05}$) compared with the UNT soil (Fig. 2b). The DL influence was the most significant. The Zn_{spw} value for the CTRL soil was higher than for all the contaminated soils, i.e. UNT: 1.09 fold, DL: 2.7 fold, and OM and OMDL: 1.4 fold ($P= 5.94E^{-07}$). In soils amended with 5% compost, with and without 5% cyclonic ashes, the Zn mobility was reduced after one week by 87% and 96% respectively compared with the Unt soil (Ruttens et al., 2006). Limestone, biosolids, cyclonic ashes, iron grit and red muds can also decrease soil Zn mobility (Brown et al. 2005). For instance the decrease in Zn_{spw} in compost- and biochar-amended soils over a 60-day field exposure was due to the Zn presence mainly in water-soluble fractions whereas the other elements such as Cu and As formed more stable complexes in the soil (Beesley et al., 2010). Conversely, Zn was immobilized in an acid soil by humic acids isolated from organic materials, whilst Cu mobility was enhanced by the same humic acids (Clemente and Bernal, 2006). Zinc is generally relatively insoluble at pH>7 (Ross, 1994), so the higher Zn_{spw} value in the CTRL soil would be related to its lower soil pH (Tab. 1). Soluble Zn concentrations in SPW from environmentally exposed green waste compost are generally low compared to other PTTE such as Cu and As (Beesley and Dickinson, 2010). The OM addition rate may influence Zn mobility and increase negatively-charged adsorption sites in the OM-treated soils (Hartley et al., 2010). In González et al. (2012), an OM addition rate of 6%, compared to 5% in our experiment, decreased Zn_{spw} while it increased at 2%.

Red mud was more effective than limestone and furnace slags to decrease shoot Zn concentration of lettuce (Lee et al. 2009). Reduced concentrations of soluble and extractable Zn in the amended soils were partly attributed to increase in the soil pH. Addition of alkaline materials such as coal fly ash and red mud also decreased Zn leaching by 99.7% and 99.6%, respectively (Ciccu et al., 2003), compared to 59% in our study. Large reductions in Zn extractability (up to 21.9%) and phytoavailability can be obtained from alkaline organic treatments, i.e. lime-stabilized biosolid and N-Viro Soil, by forming metal-carbonate precipitates (Basta et al., 2001).

The DL treatment decreased Zn_{DGT} by 45% compared to the UNT soil, being more effective than OM (11%) and OMDL (20%) (Fig. 2f). Zn_{DGT} was 8-12 times higher in the uncontaminated soil than in the contaminated ones ($P=2.30\ E^{-08}$) (Fig. 2f).

Zn_{spw} in the OM, DL and OMDL treated soils was respectively 9, 7, and 9.5 fold higher than Zn_{DGT}. This ratio peaked up to 10 for the UNT soil (29.7 and 3, µg Zn L^{-1}, respectively). The DOC correlated with Zn_{DGT} (r=0.776) in the contaminated and control soils, and Zn $_{DGT}$ with Zn_{spw} as well (r=0.57). The Zn_{DGT}:Zn_{SPW} ratio (R) was roughly 7 fold higher in the control soil than the UNT soil, the solid phase of this last one having a low capacity to resupply Zn the pore water (Tab. 5). The R value was numerically slightly lower in the UNT soil than in the treated soils and peaked in the DL soil, but differences were not significant.

In another study the Zn availability in a contaminated soil can be reduced after the incorporation of various amendments (i.e. limestone, biosolids, cyclonic ashes, iron grit and red muds), but ecosystem services were not fully restored due the residual Zn bioavailability (Brown et al. 2005). Increase in soil pH, due to organic and inorganic amendments, was suggested in line with Zn precipitation and sorption on mineral phases (Lee et al., 2009). Similar findings were reported with six cost-effective amendments ($CaCO_3$, iron grit, fly ash, manure, bentonite and bone meal) for Cd, Zn and Pb leaching and phytoavailability (Houben et al., 2012). Here, the DL incorporation into the soil slightly increased soil pH (Tab. 2), significantly decreased Zn_{SPW}, but did not change Zn_{DGT} which was initially low.

3.1.3. Soil Cr exposure

The total dissolved Cr concentration in the SPW of contaminated soils (Cr_{spw}) (in µg. L^{-1}) varied between 0.3 (OMDL) and 1.3 (OM). However this variation was not significant for the treated (OM, DL and OMDL) and untreated (UNT) contaminated soils. In contrast the control soil (CTRL) presented the

highest total dissolved Cr concentration in the SPW ($Cr_{spw)}$ (in µg. L^{-1})(2.5), this concentration was significant compared with the others contaminated soils (p=0.002)(Fig. 2c).

The Cr_{spw} value was related to TOC of the the treated and untreated contaminated soils (r=0.65). Similarly the Cr_{spw} value was related to the DOC (r= 0.82) measured in the contaminated (UNT, OM, DL and OMDL) and uncontaminated soils (CTRL). Cr_{DGT} was below the detection limit for all contaminated soils. Thus the $Cr_{DGT:}$:Cr_{SPW} ratio was Zero in the four type of soil.

The Cr mobility depends on several key-factors such as soil pH, clay mineral content, competing major ions, and complexing agents (Pantsar-Kallio et al., 2001; Seaman et al., 2001). The oxidation state of Cr in contaminated soils is an important indicator of toxicity and potential mobility. Chromium in the hexavalent Cr (VI) state is highly toxic and soluble, whereas the trivalent state Cr (III) is much less toxic and relatively insoluble. Increased Cr_{spw} in the soil after a single addition of both organic or inorganic amendment, and correlation between the soluble Cr fraction and the total and dissolved organic matter in the soil were previously reported (Kumpiene et al. 2008; Hartley et al. 2010). As Cr (III) is relatively insoluble and resistant to leaching (Palmer and Puls, 1984), the fraction we measured may include chromate (hexavalent chromium; Cr (VI)) which is more mobile under alkaline to slightly acidic conditions (e.g. pH=7.27±0.38 in the OM soil) Kimbrough et al., 1999), Higher chromate mobility may be due to the presence of other competing anions which were abundant in the compost (Jardine et al., 1999). Based on Pantsar-Kallio et al. (2001) and Seaman et al. (2001) several alkaline materials, e.g. fly ash, hydroxyapatite, and $CaCO_3$, can increase soil pH above neutral, which favor the oxidation of Cr(III) to Cr(VI), and thus enhances the Cr mobility and uptake by roots (Rai et al., 2004). This agrees with the higher Cr_{spw} value measured in the DL soil. Barnhart et al. (1997) indicated lower sorption of Cr species at higher pH. Decline in Cr_{SPW} in the OMDL soil can be due to the capacity of soil organic matter (SOM) to reduce Cr (VI) to Cr (III), which is more stable in the soil, and the negative functional groups on SOM surface which can increase Cr (III) adsorption (Bolan et al. 2003, Banks et al. 2006, Jiang et al. 2008, Adriano et al. 2001). Simultaneously, the DL addition into the OMDL soil slightly increased soil pH (Tab. 2), which may promote Cr (III) sorption by SOM.

Available Cr fraction determined by DGT was not detected in the contaminated topsoils, suggesting that soil Cr may be mainly under the Cr (III) form, which is more stable or not available to the DGT probe (Ernstberger et al., 2002). Even though chromates are not expected to bind to the chelex resin due to their anionic form, they can diffuse in the gel layer but this one was not analysed.

3.1.4. Soil As exposure

The total dissolved As concentration in the SPW ($As_{spw,}$ in µg. L^{-1}) increased from 1.8 to 4.2 in all amended soils compared to the UNT soil, and ranked as: OM > DL > OMDL > UNT (Fig. 2d). The As_{spw} peaked in both the OM soil (P=0.0003) and the CTRL soil, which had a relatively high SOM content. As_{spw} well correlated with TOC for our soil series (r= 0.69) and DOC concentrations in soil pore water (r= 0.73).

All amendments incorporated into the soil increased the As mobility in the treated soils but As_{SPW} remained below the control value (Fig. 2d). Influences of organic and inorganic amendments on the mobility, availability and phytotoxicity of soil As in the soil are controversial. Increases in As mobility after dressing of organic matter are reported (Mench et al. 2003; Hartley et al. 2009). DOM generally presents in SPW in anionic form and compost-borne anions such as phosphates may compete with As for sorption sites such as Fe oxides (Sadiq, 1997; Tate and Theng, 1980). This could enhance As leaching from the soil material (Lombi et al. 2000). In the OMDL treatment, changes in the soil pH (Tab. 2) and addition of Ca and Mg, may promote precipitation of Ca- and Mg phosphates and arsenates, metal arsenates such as those of Cu(II), which are less soluble and more stable in the neutral pH region than Ca arsenates, and may limit DOM influence. In CCA-spiked mineral soils, 92% of total As is As(V), which is less mobile and less toxic, while the proportion of mobile, toxic and bioavailable As(III)

in CCA-spiked organic soils increased to one third of the total soil As (Balasoiu et al. 2001). Other mechanisms have been suggested: (1) organic anions may block As adsorption sites (Carey *et al*., 1996) and (2) formation of soluble As-organic complexes (Chen et al. 2006; Dobran and Zagury 2006).

The DL incorporation into the contaminated soil increased the mobility of As by 2 fold (Fig. 2d), confirming previous findings (Seaman et al. 2003; Hartley et al. 2004; Mench et al. 2003). Alkaline materials such as lime, dolomitic limestone, fly ashes, and hydroxyapatites, are indeed undesirable in As-contaminated soils as they can increase As release from the soil to the SPW, due to the higher As mobility at a higher pH range. However, lime can slightly reduce As leaching in soil by 8% through possible formation of As–Ca complexes (Hartley et al. 2004). Calcium hydrogen arsenate ($CaHAsO_4$) and calcium arsenate ($Ca_3(AsO_4)_2$) can precipitate in the Ca presence under oxidizing and moderate pH conditions (Porter et al. 2004).

3.2. Biomass of primary leaves and PTTE phytoavailability

Liming and organic amendments, singly and in combination, can increase plant yields and reduce the plant exposure to Cu and Zn in metal-contaminated soils (Brallier et al. 1996; Sanchez-Monedero et al., 2004; Su and Wong, 2004; Bes and Mench 2008; Angelova et al. 2010). In our study the BLDW of Cu and Zn was numerically higher for all contaminated soils compared to the CTRL soil (Tab. 6). For the contaminated soils the lowest BLDW value was for the UNT beans: based on the UNT plants, the OM, OMDL, and DL treatments numerically enhanced BLDW by 50%, 44%, and 23% respectively (Tab. 6).

The UNT beans had higher foliar Cu, Cr, As, and Zn concentrations than the other beans (Tab. 6). Foliar metal concentrations were reduced in the OM, OMDL and DL plants compared to UNT plants, which agreed with Bes and Mench (2008). The addition of dolomite residue and to a lesser extent gypsum and phosphogypsum can also reduce both soil Cu exposure and plant Cu concentration (Garrido et al., 2005). Foliar Cu, Cr, and As concentrations of plants grow on the contaminated soils exceeded those of CTRL plants, except foliar Zn concentration which was similar. Differences between the treated soils and the UNT soils and between the contaminated soils and the CTRL soil were significant for Cu, Cr, As and Zn, except for foliar Zn concentration between contaminated and CTRL soils. Except for CTRL beans, foliar Cu concentration exceeded its upper critical threshold value (in mg kg^{-1} DW: 15-30 MacNicol and Beckett 1985; 20 Kabata-Pendias and Pendias, 2000). Foliar Cu concentration was predicted by Cu_{DGT} (r=0.78) and was correlated to soil pH (r= 0.62). Upper critical threshold values for foliar Cr, As and Zn concentrations, i.e. 1-5, 1-5, and 100-450 mg kg^{-1} DW, respectively (MacNicol and Beckett 1985; 20 Kabata-Pendias and Pendias, 2000) were just reached in the UNT plants and in overall decrease in bean growth mainly mirrored a Cu phytotoxicity confirming previous findings (Bes and Mench 2008).

Total element amount in primary leaves (µg $plant^{-1}$), so-called here mineral mass, was computed for Cu, Cr, Zn and As by multiplying foliar element concentration (µg kg^{-1} DW) and leaf biomass (µg DW $plant^{-1}$). For Cu, Cr and As, mineral masses of UNT plants were higher than those of the CTRL plants (Fig. 3). The soil treatment influenced mineral masses for Cr, Cu and As but not for Zn. This reflected a dilution effect of the biomass on Zn concentrations, i.e. foliar Zn concentration = $176.93e^{-10,61\ \text{foliar DW yield}}$ (R^2 = 0.93). Compared to the UNT plants, mineral masses of OM and DL plants decreased by 2 fold for Cr, 2.5 fold for As and ,1.3 and 3.6 for the Cu respictivelly, whereas mineral mass of Cr and Cu in OMDL plants increased by 16% and 23% recpictivelly. Differences between OM and OMDL plants were significant for Cr and Cu mineral masses. Lowest values of As_{spw} and Cr_{spw} in OMDL soil were not reflected by Cr and As mineral mass of OMDL bean (Fig. 3) the variance of Cu mineral masse was significant also between the contaminated soils (UNT, OM ,DL and OM+DL) and the CTRL soil (p=0.00005).

Table 6: The dry weight (DW) of bean primary leaves BL(BLDW) and PTTE concentrations of bean primary leaves. Values are mean ± standard deviation (n=4).

traitements	The dry weight (DW) of bean primary leaves BL(BLDW) (g DWplant^{-1})	Foliar concentrations (mg. kg^{-1})			
		Cr	Cu	As	Zn
UNT	0.06±0.01	6.89 **a**±0.54	357.50 **a**±48.81	3.29 **a**±0.56	100.10 **a**±11.22
OM	0.11±0.00	1.58 **b**±0.03	142.25 **b**±23.73	0.65 **b**±0.01	52.25 **b**±4.43
DL	0.07±0.09	2.31 **b**±0.25	211.67 **b**±20.94	1.15 **b**±0.39	75.90 **b**±22.91
OMDL	0.10±0.01	5.37**ab**±0.30	260.16 **b**±15,81	1.55 a**b**±0.07	66.68 **b**±22.23
CTRL	0.13±0.00	1.18 **c**±0.14	15.98 **c**±2.80	0.19 **c**±0.00	44.68 **c**±6.34
Anova with CTRL	**NS**	**	**	**	*
Anova without CTRL	**NS**	**	**	**	**NS**

*=<0.05, **=<0.001

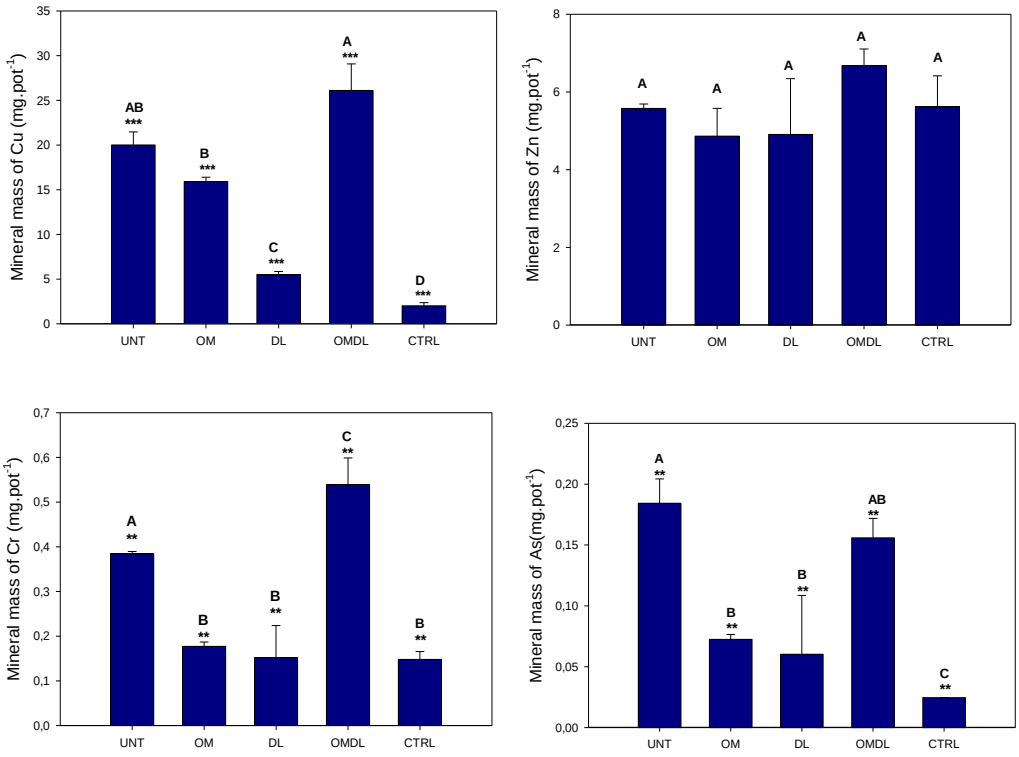

Figure 3: Mineral masses of Cu, Cr, Zn and As in the bean primary leaves

4. Conclusion

Changes in mobility and phytoavailability of Cu, Zn, Cr and As were investigated at a wood preservation site in topsoils of field plots amended with dolomitic limestone (DL) and compost (OM), singly and in combination, and phytomanaged with mycorhizal trees, after four years. The OM incorporation into the contaminated soil decreased the Cu, Cr, Zn and As concentrations of bean primary leaves to the highest extent, but only their Cr and As mineral masses were reduced. The Cu concentration in the soil pore water (SPW) increased for OM and OMDL soils whereas it slightly decreased in the DL soil. The Zn concentration in SPW significantly decreased only in the DL soil. The Cr concentration in SPW was enhanced in both DL and OM soils but slightly decreased in the OMDL

soil. The As concentration in SPW increased in all amended soils, notably for the OM soil. The addition of OM and DL, singly and in combination, decreased the available fraction of Cu in soil determined by DGT. Mobilization of Cu from the soil to the soil pore water was slower than root uptake and soil amendments reduced the replenishment of Cu in SPW. Conversely, based on the R ratio, Zn remobilization from the solid phases was enhanced in the DL soil. In overall, data suggested the influence of both pH and DOM on metals and As concentrations in the soil pore water and their availability to dwarf beans. The DL addition promoted Cu and Zn sorption in relation with pH rise, but enhanced remobilization of Cr and As, likely chromates and arsenates from Fe oxides. Nevertheless foliar Cr and As concentrations in the DL beans were lower than in the UNT beans likely due to decrease in soil Cu exposure, partial restoration of root growth and subsequent Cr and As storage in roots. Compost likely increased Cu–DOM complexes in SPW, which are less available for root uptake, and consequently enhanced the DW yield of bean primary leaves. Subsequently, decreased soil Cu exposure in both OM-amended soils allowed roots to better control metals and As uptake and promote a dilution effect in the bean plant parts.

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Chapitre 2.2.

Phytoextraction assistée des éléments traces potentiellement toxiques d'un sol contaminé au cuivre avec une rotation de cultures de tabac et de tournesol sur deux années

2.2.1. Résumé de la publication

Dans notre article précédent nous avons essayé d'évaluer la capacité de la phytostabilisation assisté par des amendements organiques et inorganiques à remédier un sol naturel contaminé fortement en Cu, situé dans le sud-ouest de la France.

Dans cet article nous avons travaillé sur le même site contaminé en cuivre et en autre ET mais cette fois sur d'autres parcelles toujours contaminées en Cu. Sur ces parcelles contaminées, on a appliqué la phytoextraction assistée par des amendements organiques et inorganique afin de tester la capacité de cette option de phytomanagement à réduire les concentrations excessives du Cu et des ET dans le sol par une rotation de cultures de tabac et de tournesol caractérisés par leurs capacité à accumuler les ET dans leurs parties aériennes.

Notre site expérimental à été divisé en quatre blocs (2 m × 10 m), e.g. Bloc n° 1: parcelle # 1 à # 10 ; bloc n° 2: parcelles # 11 à 20, et le bloc n° 3: parcelle # 21 à 30, bloc n° 4: parcelle 31 (Kolbas et al., 2011; Mench et al., 2010). Ces blocs (# 1-3) ont été amendés en Mars 2008 avec le compost (OM) (5% w/w) et la dolomie (DL) (0,2% p/p). Le bloc # 4 n'a pas été traité et a été considéré comme une seule parcelle (UNT n ° 31). Ensuite un terrain non contaminé (1 m × 2 m) situé à 18 km du site, de la même terrasse alluviale et avec un type de sol similaire, a également été échantillonné comme un sol de contrôle (CTRL). Sur ces parcelles contaminées une rotation des cultures de tabac et de tournesol à été mis en place pour tester leurs capacités à diminuer les concentrations excessives des éléments traces potentiellement toxiques (PTTE).

En Avril 2010, trois échantillons de sol (1,5 kg sol) ont été recueillis dans les parcelles amendées (20, 8, 14, 13, 26 et 30), la parcelle non-traitée (31) et le sol de contrôle (CTRL). Ces parcelles ont été sélectionnées parce qu'elles sont caractérisées par des paramètres physico-chimiques identiques mais avec la présence de concentrations croissantes de cuivre dans les sols, ce qui offrent une occasion unique d'étudier l'effet des concentrations totales croissantes du Cu sur la mobilité, l'intensité d'exposition du sol et la phytodisponibilité de Cu et des autres ET dans les sols. Ces concentrations augmentent comme suit: 163, 268, 382, 518, 753, 1170 mg Cu / kg respectivement pour les parcelles, et 832 mg Cu / kg pour la parcelle non traitée. Les trois échantillons ont été pris de la couche 0-25 cm du sol de chaque parcelle ; ensuite ces échantillons ont été mélangés, séché à l'air et tamisés (<2 mm, filet de nylon) et combinés pour produire un échantillon de sol composite (1 kg).

Les concentrations totales dissoutes des PTTE ont été déterminés dans l'eau interstitielle du sol (SPW) échantillonnée par un capteur Rhizon. L'intensité de l'exposition du sol a été évaluée par la technique du DGT (peeper-gel) qui a permis de mesurer les concentrations disponibles des PTTE. La phytodisponibilité des PTTE a été caractérisée par la croissance des haricots nains en pot et l'analyse de leurs concentrations foliaires en PTTE. Toutes ces analyses sont réalisées en laboratoire sur un échantillon du sol de chaque parcelle où ont été appliquées les techniques de phytoextraction assistée. L'échantillonnage a été réalisé deux années consécutives. Les analyses élémentaires sont réalisées par ICP-MS (Element 2, Thermofischer).

Nos résultats ont montré que les plantes accumulatrices (tournesol et tabac) qui ont été cultivées sur le site ont réduit les concentrations de Cu, Zn, As et Cr significativement de 2010 à 2011 de 61%, 58%, 60% et 40% respectivement. Nous avons trouvé que les concentrations totales de Cu dissous mesurées dans le SPW du sol contaminé et sol de contrôle (CTRL) en 2010 et 2011 a été beaucoup plus élevé que pour les autres PTTE (Zn, As, Cr). Cette concentration de Cu dissous dans le SPW a augmenté en 2010 et 2011 linéairement avec la concentration totale du Cu dans le sol en 2010 et 2011. Les concentrations dissoutes du Zn, Cr et As dans les sols contaminés amendés ont diminué par rapport au sol sans amendement et le sol de contrôle (CTRL). Ces PTTE ont montrés aussi une bonne corrélation avec le pH du sol, la concentration en carbone dissous (DOC) des SPW et la matière organique totale mesurée dans ces sols. On a constaté aussi que les concentrations disponibles du Cu et Zn mesurées par le DGT ont diminué considérablement de 2010 à 2011.

Nous avons également trouvé que l'intensité de l'exposition du sol pour tous les PTTE a diminué de façon significative après deux années de rotation des cultures. La phytodisponibilité de Cu étudiée par la concentration dans les feuilles de haricots (BL) ou la masse minérale du Cu, a augmenté aussi linéairement avec les concentrations totales du Cu dans le sol.

Nous pouvons donc conclure que l'application de la phytoextraction assistée sur un sol naturel contaminé en PTTE et spécialement en Cu à été capable de diminuer les concentrations dissoutes des PTTE dans la solution du sol, et diminuer également les fractions disponibles des PTTE. Cette option de phytomanagement a également la capacité à réduire les concentrations mesurées dans les feuilles de haricot et l'intensité de l'exposition du sol pour tous les PTTE.

2.2.2. Article 2 (en préparation)

Assisted phytoextraction of potentially toxic trace elements in copper contamined soils with a crop rotation of tobacco and sunflower over two years

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Keywords: Phytoextraction, Sunflower, Tobacco, Soil pore water, potentially toxic trace elements, Soil exposure intensity, Phytoavailability

Abstract: Copper-contaminated soils were managed with assisted phytoextraction in 7 field plots at a wood preservation site to evaluate the decontamination efficiency of tobacco and sunflower. The mobility and bioavailability of four potentially toxic trace elements (PTTE) i.e. Cu, Zn, Cr and As were investigated in these soils with a crop rotation of tobacco and sunflower and with the incorporation of compost (OM) and dolomitic limestone (DL). Sub-plots with the presence of increasing concentrations of copper in their soils but identical physico-chemical parameters were selected together with a control uncontaminated plot. They provide a unique opportunity to investigate the effect of the total soil concentration on the mobility, availability and phytoavailability of Cu. A cultural cycle of two years was applied to these sub-plots, alternating between several species of sunflower and tobacco, to examine the effectiveness of the phytoextraction and the ability of these species to reduce the concentrations of the PTTE in these contaminated soils. Total PTTE concentrations were determined after two years of crop rotation in the soil pore water (SPW) sampled by Rhizon soil moisture samplers. The soil exposure intensity was assessed by DGT (diffusive gradient in thin films) probes. The PTTE phytoavailability was characterized by growing dwarf beans on potted soils and analyzing their foliar PTTE concentrations. Total dissolved Cu concentrations in the SPW increased with the total soil concentration of Cu. The crop rotation of sunflower and tobacco significantly decreased the total dissolved concentrations of Cu, Zn , Cr and As in the SPW. The soil exposure intensity for all PTTE also declined significantly after two years of crop rotation. The phytoavailability of Cu as studied by the BL concentrations or the mineral mass of PTTE increased with the total soil concentrations. The phytoavailability of the other PTTE also decreased after two years as the foliar PTTE concentrations together with the mineral masses showed lower values.

Abbreviations:

Chromated copper arsenate(CCA), control soil (CTRL),copper mineral mass(CuMM), CuTOT: total soil Cu, DOM: dissolved organic matter, compost made of poultry manure and pine bark chips (CPM), dolomitic limestone (DL), untreated (UNT), potentially toxic trace elements (PTTE), diffusive gradient in thin films (DGT), soil pore water (SPW), organic matter (OM), soil organic matter (SOM), dissolved organic matter (DOM), electrical conductivity (EC), total organic carbon (TOC), dissolved organic carbon (DOC), bean leaves (BL), dry weight (DW), dry weight of primary leaves (BLDW), [Cu_{spw}] concentration of Copper in the soil pore water(SPW). Mineral mass (MM), Metals concentration in the planet (PLT), concentration of available copper in the soil [Cu_{DGT}]

1. Introduction

Excessive concentration of potentially toxic trace elements (PTTE), such as Cu, Zn, Cr and As in soils poses significant hazard to human, animal and plant health and to the environment in general. Due to the high persistence of PTTE and their potential toxicity, soils contaminated with these elements are an environmental problem that requires an effective and economically affordable solution. In France there are about 680 Cu contaminated sites referenced by the national authority (Basol 2011). In many cases, the use of Cu-based preservatives (e.g. Cu sulphate and CCA) is involved (ADEME 1998). Consequently, topsoils are Cu-contaminated with ecotoxicological effects on flora and fauna, dire consequences for vegetal communities, the food chain quality and water supplies (Bes et al., 2008; Bes and Mench, 2008).

Although a number of techniques have been developed to remove PTTE from these contaminated soils like pneumatic fracturing, soil flushing, solidification, vitrification, electrophoresis, chemical reduction, soil washing and excavation, many sites remain contaminated because economic and environmental costs to clean up those sites with the available technologies are too high. Since then, scientists have been in search of some innovative, eco-friendly and low cost alternative technologies. One of them is phytoremediation which includes the use of plants to clean and cure the environment. Plants have been indeed known for their property to absorb, accumulate and detoxify the impurities present in the soil, water and air through various physical, chemical and biological processes (Hooda, 2007). Phytoremediation, a fast-emerging new technology for removal of toxic trace elements, is cost-effective, non-intrusive and aesthetically pleasing. It exploits the ability of selected plants to remediate pollutants from contaminated sites by extraction, volatilization, stabilization and rhizofiltration (Wani et al; 2012).

In particular the phytoextraction technique uses plants to extract and translocate metals from the soil to the harvestable parts of plants. The aim of this technique is to reduce the concentration of PTTE in contaminated soils to regulatory levels within a reasonable time frame. Plants used for extraction of PTTE from contaminated soils must be tolerant to PTTE, adapted to the local soil and climate characteristics and able to take up a large amount of PTTE (Keller et al., 2003). Phytoextraction will be more economically feasible if, in addition to PTTE removal, plants also produce biomass with an added value (Vassilev *et al*., 2004). For example, energy crops (oilseed and willow), fibers and fragrance producing plants could be used to recover these valuable products (Schwitzguébel *et al*., 2002). Generally, two approaches have currently been used to reach this goal: 1) the use of plants with exceptional, natural metal-accumulating capacity, the so-called hyper accumulators, and 2) the utilization of high-biomass crop plants, such as maize, tobacco and sunflower, compensating moderate metal accumulation by a high biomass yield (Mench *et al*., 1989; Kumar et al., 1995; Herzig et al., 2003; Vassilev et al., 2004; Lombi et al., 2000).

Additionally phytoextration can be applied on contaminated soils in combination with soil conditioners (so-called assisted phytoextraction). Merging together the cultivation of non-food crops for the supply of plant-based feedstock, amelioration of metal-contaminated soils, and risk management is developing at the field scale (Vangronsveld et al., 2009; Meers et al. 2010; Mench et al., 2010; Kolbas et al 2011). The phytoextraction technique using Sunflower (*Helianthus annuus* L.) is characterized by several advantages which are its ability to accumulates moderate PTTE concentrations and extract PTTE such as Zn, Pb, Cd and Cu from the soil due to its high biomass production (Lombi et al. 1998; Yankov and Tahsin 2001; Madej´on et al. 2003; Soudek et al. 2007; Marchiol et al. 2007; Nehnevajova et al. 2005, 2007b, 2009a; Vangronsveld et al. 2009). It can provide financial returns through oilseed and biomass production and can be included in a sustainable crop rotation promoting soil development processes, nutrient cycles, and microbial community and soil ecosystem functions with either or no acceptable residual pollutant linkages (Faessler et al. 2010a; Nehnevajova et al. 2009b; Shehata and Razek 2011;

Tahsin and Yankov 2007). Additionally copper phytoextraction and oilseed production for biodiesel through sunflower cultivation may be an option to minimize the pollutant linkages and achieve some financial returns at a wood preservation site.

The accumulation of PTTE in the shoots of sunflower depends on several factors related to plants such as PTTE absorption by roots, PTTE translocation from roots to shoots, differences in rooting depth and density, impacts of pests, pathogens and herbivores (Vassilev *et al*., 2002). Other reactions associated with soil which controls the mobility and the availability of PTTE are sorption/desorption reactions as well as chemical complexation with inorganic and organic ligands and redox reactions, these reactionss are of great importance in controlling their bioavailability, leaching and toxicity of PTTE. These reactions are affected by many factors such as pH, nature of the sorbents, presence and concentration of organic and inorganic ligands, including humic and fulvic acid, root exudates, microbial metabolites and nutrients (Violante et al.,2010).

The main thrust of this work was to investigate the ability of several types of sunflowers and tobaccos on Cu-contaminated soils with and without the addition of compost (5%) and dolomitic limestone (0.2%) to: 1) extract the PTTE from the soil, 2) to decrease the total dissolved soil pore water (SPW) concentrations and 3) to decrease the available concentrations of PTTE in this soil under real field conditions. Our selection of plots was because the presence of increasing concentrations of copper in their soils; these concentrations are increasing as follows: 163, 268, 382, 518, 753, 1170 mg Cu/kg respectively to the plots, and 832 mg Cu/kg for the untreated plot. Total dissolved concentrations of PTTE (Cu, Zn, Cr, and As) were quantified in SPW sampled by Rhizon moisture probes to assess the PTTE mobility. The soil exposure intensity of Cu and Zn was determined by DGT probes. The PTTE phytoavailability was characterized by cultivating dwarf beans and analyzing their primary leaves for their elemental content.

2. Material and Methods

2.1. Site and Soils

The wood preservation site (6 ha) is located in the Gironde County (44°43'N; 0°30'O), southwest France. These hectares have been used for over a century to preserve and store timbers, posts and utility poles and various Cu-based salts were successively utilized (Mench and Bes, 2009). The industrial facility dates back to 1846 with the building of a railway (France, X 1973.15; Y 376.8). In 1950 Creosote, CuSO4, and standard CCA-type C have been successively used as wood preservatives, but only CCA has been used since 1988.

Two ha from the six remain in industrial activity for wood preservative treatment, recycling of treated wood and frame production. This site has a sandy soil, i.e. 85.8 % sand, 5.9 % clay, 8.3 % silt as it is developed on an alluvial soil in terrace (Fluviosol) containing alluvial materials from the Garonne River combined with wind deposits (BRGM, 1978). It contains 1.6 % OM, and C/N 17.2, with generally a low CEC (3.5 cmol. kg^{-1}) (Bes, 2008). The characterization of the site demonstrated that copper presents the major contamination of topsoils, which showed considerable spatial variation (65–2400 mg Cu kg^{-1} soil DW), then, the total soil As and Cr which present environ 10–53 mg of As and 20–87 mg of Cr kg^{-1} in topsoil's, remained relatively low in all soil layers (Bes 2008). This site is divided into fifteen sub-sites labeled from A to E and P1 to P10 according to past and present activities, plant communities, employees' evidence, aerial and archived photos and site history (Mench and Bes 2009; Bes et al. 2010; Kolbas et al., 2011).

Our experimental site is divided into four blocks (2 m × 10 m), i.e. block #1: plots #1 to #10 block #2: plots #11 to 20, and block #3: plot #21 to #30, block #4: plot 31 (Kolbas et al. 2011; Mench et al. 2010). These blocks (#1–3) were amended in March 2008 with the compost made from poultry manure and pine bark chips (CPM) (5% w/w, Orisol, Cestas, France) and dolomite DL (0.2% w/w). Block #4 remained untreated and was considered as a single plot (UNT #31). Soil

amendments were carefully mixed in the topsoil (0-0.25 m) with a stainless spade. Each amended block was divided into 10 sub-plots (1 m × 2 m) (Figure 1). An uncontaminated plot (1 m × 2 m) from a kitchen garden (CTRL) located 18 km from the site, from the same alluvial terrace and with a similar soil type, was also sampled.

An cultural cycle of two years has been applied to these sub-plots in 2010 and 2011, alternating between several species of sunflower and tobacco, to examine the effectiveness of the phytoextraction and the ability of these species to reduce the concentrations of potentially toxic trace elements (PTTE), i.e. Cu, Zn, Cr, and As in this contaminated soil (Kolbas et al., 2011). In April 2010, three soil samples (1.5 kg soil FW) were collected from the amended plots that have the following number 20, 8, 14, 13, 26, and 30, one sample from the untreated one (31) and one sample from the control soil (CTRL). Our selection of these plots in particular was because the presence of increasing concentrations of copper in their soils; these concentrations are increasing as follows: 163, 268, 382, 518, 753, 1170 mg Cu/kg respectively to the plots, and 832 mg Cu/kg for the untreated plot. The three samples from the 0–25 cm soil layer of each plot were mixed with a stainless spade, and combined to produce a composite soil sample (1 kg FW) which was air-dried, sieved (<2 mm, nylon mesh) and manually homogenized.

The same soils sampled in 2010 were resampled in 2011 to investigate the effects of phytoextraction on the mobility, soil exposure intensity and phytoavailability of PTTE in the soil.

2.2. Soil pore water sampling and analysis

2.2.1. Measurements of trace metals in the soil pore water (SPW)

For measuring the trace metals in the soil pore water (SPW), one kg of air-dried soil from each plot (n=8, 13, 14, 20, 26, 30 and 31) was potted (1.3 L) after sieving (2 mm). Additionally an uncontaminated control (CTRL) soil from a garden (Gradignan, France) of the same alluvial terrace was taken. These soils were watered with deionised water, and daily maintained at 80% of the water field capacity (WHC) (10% of air-dried soil mass) for 15 days. After 15 days three Rhizon MOM moisture samplers (Eijkelkamp, The Netherlands) were inserted with a 45^{o} angle into each potted soil. The capped end of each Rhizon was inserted into each potted soil during filling. A syringe needle was connected to the female lock and inserted into a 10 mL glass vacuum tube, for extracting the SPWs by vacuum (Cattani, 2006). Before putting the samplers in the soils, the SMS devices were previously cleaned with 5% HNO_3 and then washed twice with *deionised water*. Each device was then let under vacuum for 24 hours for collecting the soil pore water (~ 10 mL). The SPWs were stored at 4°C. A fraction of each soil pore water was acidified to 0.1 mol/L HNO_3 for measuring the concentrations of Fe, Mn, Cu, Zn, Cr, and As in the samples by HR-ICP-MS (Element 2, Thermofischer). The rest of the solutions was kept for the following analyses: pH, electrical conductivity (EC), dissolved organic carbon (DOC) which was determined by a carbon analyzer (Shimadzu© TOC 5000A), whereas concentrations of major cations (Na^+, K^+, Ca^{2+}, and NH_4^+) and anions (NO_3^-, SO_4^{2-}, and Cl^-) were determined by ionic chromatography (Dionex ICS-2000, Sunnyvale, CA using CS16A for measuring cations and AS17 for anions columns respectively). The total organic carbon (TOC) was determined in the soil by the Rock-val. This instrument uses a ramped temperature pyrolysis technique whereby a small amount of soil sample (70 -100 mg) is heated in an inert atmosphere (helium or nitrogen) and also combusted with air to obtain several key geochemical parameters such as the total organic carbon (TOC).

2.2.2. Soil exposure intensity

For characterizing the soil exposure intensity to PTTE (Cu, Zn and Cr) and measuring available concentrations in the soils, we have used the Diffusive Gradients in Thin-films (DGT) devices which have an active surface area of 3.14 cm^2, this device consist of a plastic base which contain: three layers: the first one is a 0.45μm filter; the second layer is a diffusion layer which consists of a

polyacrylamide gel layer and the third one a polyacrylamide gel layer that incorporates a Chelex-100 resin that binds strongly the labile trace metal species (Davison et al., 2000; Ernstberger et al., 2002).

In each pot two standards cylindrical DGT units were manually inserted for 24 h directly into the humid topsoil (80%). DGT continuously accumulates metals on the resin gel during deployment. The total mass of each metal (*M*) accumulated per unit area over the deployment time (*T*) is given by integrating the flux over the deployment time (eq. 1):

$$M = \sum_{t=0}^{T} F(T)dt \qquad \text{(eq. 1)}$$

The total mass of each metal *(M)* is determined analytically through the area exposed to the solution *(A)* by measurement of the eluent concentration (*C*e) after elution of the resin gel (volume, *V*gel) with 1 M HNO_3 (volume, *V*HNO3),

$$M = C_e\,(V_{HNO3} + V_{gel})/fe \qquad \text{(eq. 2)}$$

With f=0.8 for Cu, Cr and Zn.

The strong binding of metals in the resin gel leads to the creation of a linear concentration gradient in the diffusive gel. This gradient depends on several factors such as the interfacial concentration of labile trace metal species, C_i and the thickness of the diffusion layer, Δg (cm), All these factors determines the flux, $F(t)$, of metal from the soil to the resin-gel according to Flick's first law (eq. 3):

$$F(t) = \emptyset d D d \frac{Ci(t)}{\Delta g} \qquad \text{(eq. 3)}$$

where $\emptyset d$ is the porosity of the diffusion gel and Dd (cm^{-2} s^{-1}) is the diffusion coefficient of the labile trace metal species in the diffusion layer. The diffusion coefficients were taken from Zhang et al. (2001). The averaged interfacial concentration, C_{DGT}, or available concentration can be calculated from *M* (eq. 4)

$$CDGT = \frac{M\Delta g}{\emptyset d D d} \qquad \text{(eq. 4)}$$

For each metal, the division of the available concentration (C_{DGT})on the total concentration measured in the SPW, C_{SPW}, give the ratio, *R*, which indicates the extent of the depletion of soil pore water concentrations at the DGT interface (eq. 5)

$$R = C_{DGT}/C_{SPW} \qquad \text{(eq. 5)}$$

For each metal, the mass accumulated in the resin-gel layer was determined after extraction of the resin gel by 1 mL of HNO_3 (Suprapure, MArck Darmstadt, Germany) 5% for 24h. This solution was further diluted 10 times with HNO_3 2% and analyzed by HR-ICP-MS (Element 2, Thermo Fischer) for determining metal concentrations (Cu, Cr and Zn).

2.3. Germination tests

Germination tests were applied on the soil samples of 2010 and 2011 to assess whether the use of phytoextraction technology was able to reduce the concentrations of phytoavailable PTTE in these plots. To achieve this objective two replicates per soil were prepared, and four seeds of dwarf beans (*Phaseolus vulgaris*) were sown in each one after extracting the solution of soil for measuring the mobile and available fraction of metals in the soils. These seeds were cultivated for 18 days in controlled conditions (16 h light/8 h darkness regime, 23/15C° day/night temperature). The soil moisture was maintained at around 50% of the field water capacity with additions of deionized water after weighing. Then the moisture of soil was raised to 80% at the beginning of seed germination. At harvest, the wet and dry weight (DW) of bean primary leaves (BLDW) was determined after drying at 70°C.

Aliquots of primary leaves (BL) were weighed (35-150 mg) directly into Savillex PTFE 50mL vessels. 2 mL milliQ H_2O and 2 mL of concentrated HNO_3 were added and heated open at 65°C for 2 hours. Then caps were closed and containers were left overnight at 65°C (12-14h). Thereafter, 0.5mL of H_2O_2 (30%) was added to each

sample and containers left open at 75°C for 3 hours. Then 1.5+/-0.5 mL HF (48%) was added to each sample, caps closed and containers left at 100°C overnight. Containers were opened and kept at 120°C for 4-5 hours evaporating to dryness, taken off heat, 1mL HNO_3 +5mL H_2O + 0.1mL H_2O_2 were added to each, gently warmed up (65 °C) and after cooling down made up to 50mL. Mineral composition of the digests was then determined by ICP-MS (Varian 810-MS) and elemental concentrations of the BL were determined for Cu, Cr, As and Zn. All foliar element concentration is expressed in mg kg^{-1} DW. The mineral mass of each PTTE in BL was computed based on their elemental concentrations and the BL DW.

2.4. Statistical analysis

The total SPW concentrations, DGT concentrations, R ratios, foliar element concentrations, foliar mineral masses of elements and leaf DW yields were statistically analyzed by ANOVA (Statistica) to evaluate the effects of the increasing concentration of Cu on the mobility, availability and bioavailability of several PTTE such as Cu, Cr, Zn, As. All analytical determinations were performed in two replicates. Differences were considered significant if p-value was $p < 0.05$. R2 was the determination coefficient of the linear regression curve.

3. Results and discussion

3.1. Soil and soil pore water characteristics

The physico-chemical characteristics (pH, DOC, EC, cations and anions) of the SPW extracted from the contaminated soils treated with (2% DL+5% CPM), which containing increasing total soil concentrations of Cu (Table 1), from the untreated soil and from the control soil, and the TOC in the same soils were determined in 2010 and 2011. Total soil concentrations were in the common range of French sandy soil for Cr, As and Zn but the total soil Cu concentration was in excess for these coarse sandy soils *i.e.* 35 mg Cu kg^{-1} (Tab. 1, Baize et al., 1997; 2002). In the site the total soil content (in mg. kg^{-1}) varied between 4.8–8.6 for As, 15.8–22.5 for Cr and 35–98 for Zn (Tab. 1). Total soil As, Cr, and Zn concentrations did not differ very much between the plots. Globally, Cu was the main contaminant of the plot topsoil's.

The measurements revealed that the pH decreased slightly from 2010 to 2011(Tab. 2). The untreated soil has a given pH value lower than the others soils in 2010 (6.48) and 2011 (5.88), while the control soil recorded the highest soil acidity compared to treated and untreated soils in 2010 and 2011 (7.95) and (7.77). We did not notice a lot of changes between most of DOC and TOC values in 2010 and 2011. However we can observe that the CTRL gave the highest values of DOC (124,2 mg. L^{-1}) and TOC(3,94 %) in 2011 compared with other soils and these concentrations were much higher than the concentration which was given in 2010 of DOC (63,06 mg. L^{-1}) and TOC (0,63%), While the UNT soil provided the lowest TOC compared with the others soils. With regards to EC we can see that the values measured in 2011 were higher than those measured in 2010 for all samples except T20 (1923 µS. cm^{-1}), T8 (1111 µS. cm^{-1}) which presented values in 2010 higher than those presented in 2011(1423, 1011 µS. cm^{-1}) respectively (Tab. 2).

Our results agree with that reported by Kolbas et al., 2011, who worked in the same site and tested the effect of assisted phytoextraction on the soil properties. They found that UNT provided the lowest pH, DOC and TOC, while the CTRL soil that provided the highest pH and DOC compared with the others amended soils. Singh, et al. 2007 investigated also the effect of organic amendment in assisted phytoextraction of arsenic by Vetiveria Zizanioides from a contaminated soil. They reported that after one year of phytoextraction, the control soil provided the highest pH and TOC compared with the other soils. We can also notice that the changes in the concentration of anions from 2010 to 2011 were increasing for Cl^- and $SO4^{-2}$ and decreasing for $NO3^-$ and $PO4^{-3}$ which we did not detect in 2011. In contrast the concentrations of all cations (Na^+, Mg^{+2}, K^+, Ca^{+2}, Fe^{+2}) increased in 2011 in comparison to 2010. Additionally we could not measure any concentrations of Mn^{+2} in the two years.

Table 1: Total PTTE concentrations in the topsoil's (2010) adapted from Kolbas et al., 2011

	Total PTTE concentration in the topsoils (mg. kg^{-1})			
plot#	**Cu**	**Zn**	**Cr**	**As**
T20	163	73,8	17,7	6,54
T8	268	58,1	16,4	5,33
T14	382	50,1	17,4	5,61
T13	518	50,7	19,4	6,9
T26	753	39	16,2	5,35
T31(UNT)	832	35,2	18,8	5,73
T30	1170	59,8	16,3	6,19
Control soil[a]	21	51	18	3,6
BL[b]	3.2–8.4	17–48	14.1–40.2	1–25

[a]Control soil (Mench and Bes 2009).
[b]BL: background levels; for As, mean of frequent As values for all French soil types; for other elements, median and upper whisker values in French sandy soils (Baize 1997; Baize and Terc´e 2002). Bold letters indicate concentrations exceeding (>10%) background levels in French sandy soils.

3.2. Mobility of PTTE

Concentrations of PTTE in SPW are relevant indicators of plants exposure to metals such as Cu for phytoextraction studies (Sauvé et al. 1997; Sauvé 2003; Tandy et al. 2006; Forsberg et al. 2009). SPW collected from the treated, untreated and CTRL plots showed a clear difference in the total dissolved concentration of PTTE between 2010 and 2011. We can observe that the accumulator plants (Sun flower, tobacco) that have been cultivated on the site reduced the concentrations of Cu, Zn, As, Cr significantly from 2010 to 2011 by nearly 61%, 58%, 60% and 40% respectively (Fig. 1).

Total dissolved Cu concentrations measured in the SPW of the contaminated and the control soil (CTRL) in 2010 and 2011 was much higher than the other PTTE (Zn, As, Cr) (Fig. 1). The total dissolved Cu concentrations in the SPW for the contaminated soils in 2010 and 2011 was far greater than for the control soil (CTRL) which presented 203.12 µg.L^{-1} in 2010 and 115.13 µg.L^{-1} in 2011(Fig. 1.a). The total dissolved Cu concentrations in the SPW increased in 2010 and 2011 linearly with the total soil concentration of Cu in 2010 and 2011 (R^2 > 0,9). However the distribution of the values are more scattered at a high total soil Cu concentration, which may reflect the influence of other factors, e.g. organic matter content, soil pH etc..

Our results are in agreement with Kolbas et al., 2011, who tested the efficiency of phytoextraction to reduce the high concentration of Cu in the soil. They also found also that total dissolved Cu concentrations in the SPW increased linearly with the total soil concentration of Cu. Focusing on the distributed samples, we can also notice that the samples can been divided into two groups, the first group with low total concentrations of Cu and the second group with high concentration of Cu in the soil. In the two groups we can notice that a very strong correlation (R^2 >0.7) was observed between the total dissolved concentrations of PTTE of the SPW and the total metals concentrations in the top soil. However the relation between the two groups together was relatively very weak (R^2 > 0.2) compared to their correlation individually.

The total dissolved Zn concentration in the treated contaminated soils decreased compared with the untreated contaminated soil and the control soil. The variation between the treated and untreated soil and the control soil was significant in 2010 and 2011 ($p<0.0001$) (Fig. 1.b). The total dissolved Zn concentration in the SPW showed a good correlation with the SPW pH (R^2= 0.77), DOC and the TOC of soil (R^2> 0.5) measured in 2011 but did

not show any correlation with the other soil and SPW parameters. Our results agree with Meers et al., 2005, who tested the effect of two types of amendments (ethylene diamine tetraacetate (EDTA), ethylene diamine disuccinate (EDDS)) on the mobility and availability of trace elements in phytoextracted contaminated soil. They found that the mobility of metals was reduced in the plots treated with EDDS compared with that treated with EDTA and control soil. Tandy et al., 2005 investigated the influence of the biodegradable chelating agent EDDS on the uptake of essential (Cu and Zn) and found that Cu and Zn concentrations in the plant shoot were reduced in the treated soils compared with the control one.

Fig. 1.c and d show that in the most cases, the addition of amendment to the soil reduced the total dissolved Cr and As concentrations in the SPW extracted in 2011 significantly ($p<0.0001$) compared with the untreated and control soils ($p=0.00004$ in 2011). However this effect was not significant in 2010. The correlation between the total dissolved Cr and As concentrations in the SPW and the total Cr and As concentration in the top soil was very weak in 2010 and 2011. However the total dissolved Cr concentration in the SPW was strongly correlated to the pH, DOC and TOC measured in 2011($R^2> 0.7$) and to the total concentration of Cu in the top soil in 2010 ($R^2= 0.68$). Moreover the proportion of the total dissolved concentrations in the SPW compare to the total soil concentration was very low in most cases (<0.012% of Cr in 2010, <0.006% of Cr in 2011) for the Cr; (<0.10% of As in 2010, <0.04% of As in 2011) for the As. We also noticed that this proportion was not related to the total concentrations of these elements in the soil. .

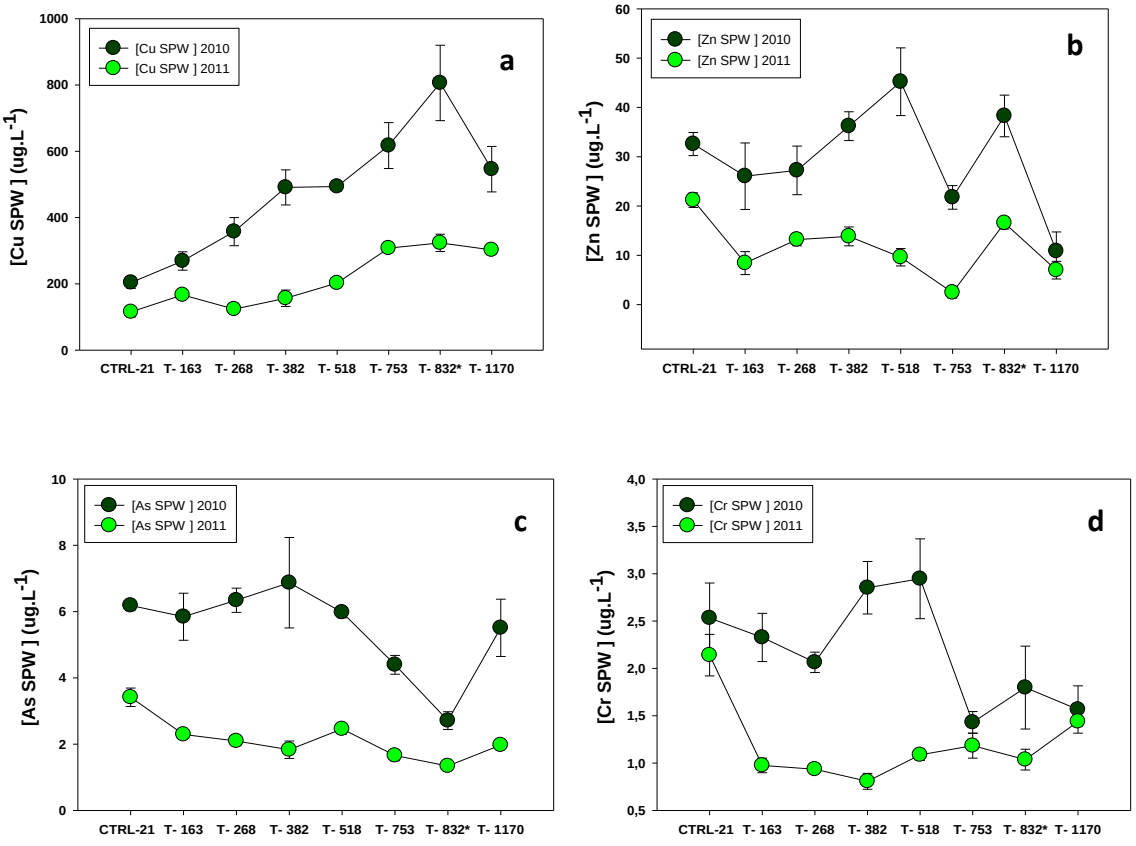

Figure 2: SPW concentrations of Cu, Zn, As and Cr measured in 2010 and 2011.

Table 2: Chemical characteristics of the SPW and soils (2010 and 2011)

Soil			soil solution										
2010													
					Anions (mg. L^{-1})				**Cations (mg. L^{-1})**				
Soil 2010	**TOC %**	**pH**	**EC ($\mu S.\ cm^{-1}$)**	**DOC (mg. L^{-1})**	**Cl^-**	**$NO3^-$**	**$SO4^{-2}$**	**$PO4^{-3}$**	**Na^+**	**Mg^{+2}**	**K^+**	**Ca^{+2}**	**Fe^{+2}**
T20	0,89	7,57	1923	66,24	10,03	326,8,	17,37	487,88	11,69	17,4	13,04	105,24	0,522
T8	1,04	7,67	1111	47,664	7,83	230,27	14,36	587	6,98	16,64	8,67	72,13	0,96
T14	0,86	7,65	1076	81,3	10,21	454,86	20,84	624,7	12,25	32,87	11,75	93,23	0,659
T13	1,01	7,79	1404	47,646	7,42	99,85	7,53	877,49	6,61	6,55	3,45	35,29	0,497
T26	1,2	7,66	1006	45,012	7,09	182,03	20,1	658,43	7,53	16,73	9,3	61,67	0,23
T30	1,07	7,74	1021	104,1	21,37	1359,12	51,4	635,41	21,95	49,16	37,77	355,6	0,471
T31(unt)	0,59	6,48	839	46,356	12,27	214,51	12,75	638,86	13,48	4,07	8,36	87,64	0,212
CTRL	0,63	7,95	574	63,06	9,58	567,56	13,92	737,42	8,6	23,21	49,74	113,71	2,542
2011													
T20	1,18	7,17	1423	67,86	71,38	*ND*	478,39	228,47	47,97	44,77	21,03	162,88	ND
T8	1	7,27	1011	55,23	107,52	*ND*	624,59	169,72	61,17	48,44	16,1	195,39	0,057
T14	1,01	7,25	1676	39,51	86,75	*ND*	607,55	183,89	51,65	54,96	27,98	252,07	ND
T13	1	7,39	1504	46,2	101,5	*ND*	578,15	127,12	57,99	41,18	17,84	188,53	0,048
T26	0,96	7,26	1206	48,45	72,46	*ND*	355,55	119,54	44,97	40,26	13,9	129,85	0,01
T30	1,2	7,34	1321	34,65	78,08	*ND*	479,01	158,06	43,57	32,78	31,3	139,36	0,042
T31(unt)	0,72	5,88	1339	35,37	98,2	*ND*	449	64,87	44,41	9,08	18,81	155,08	ND
CTRL	3,94	7,77	1074	124,2	72,9	*ND*	255,62	49,98	68,44	18,14	99,44	110,34	0,201

3.3. Soil exposure intensity of PTTE

Figure 2 shows the impact of the increasing concentrations of Cu in the top soils and the effect of aided phytoextraction on the amount of available concentrations of Cu and Zn. It can be seen that the available concentrations for both metals declined significantly from 2010 to 2011. The decline in the average of available concentrations of Cu in all plots decreased from (48 µg.kg^{-1}) of Cu in 2010 to (21 µg.kg^{-1}) of Cu in 2011, and from (16.3 µg.kg^{-1}) of Zn in 2010 to (4.5 µg.kg^{-1}) of Zn in 2011. The concentrations of available Cu and Zn in the treated soils were relatively lower than the concentrations measured in the untreated soil in 2010 and 2011 and lower than that measured in the control soil (CTRL) in the case of Zn. We did not measure any concentration of available Cr in 2010, neither in 2011. The proportion of the available concentrations of Cu and Zn to their total concentrations in the top soil was very low in most cases (<0.01% of Cu in 2010, <0.007% of Cu in 2011) for the Cu, and (<0.1% of Zn in 2010, <0.4% of Zn in 2011) for Zn. We can also notice that increasing total concentrations of Cu in the soil gave also and increasing concentration of available Cu in 2010 and 2011. A strong correlation could be seen between the concentrations of available Cu and the total soil concentration of Cu , the total dissolved Cu concentration in the SPW and with the soil solution pH in 2011 ($R^2 > 0,6$). In contrast we did not find any correlation between the soil and solution parameters and the available Cu in 2010. Additionally a good correlation was observed between the available concentrations of Zn and the total concentration of Cu in the top soil in 2011 (R^2= 0.5) and with the pH in 2010($R^2 = 0,59$) (Tab. 3,4).

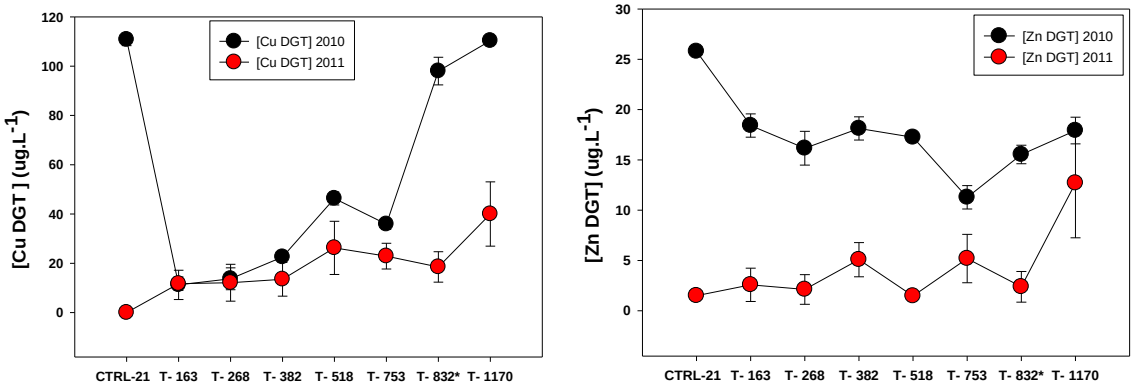

Figure 2: Cu and Zn concentration in the soil pore water measured in 2010 and 2011

Table 3 . Pearson's correlation coefficients between soil and soil pore water parameters (2010)

2010	pH	CE (µS. cm^{-1})	DOC (mg. L^{-1})	TOC (%)	[Cr SPW] (µg.L^{-1})	[CuSPW] (µg.L^{-1})	[Zn SPW] (µg.L^{-1})	[As SPW] (µg.L^{-1})	[Zn DGT] (µg.L^{-1})	[Cu DGT] (µg.L^{-1})	[As TOT] (mg.Kg^{-1})	[Zn TOT] (mg.Kg^{-1})	[Cu TOT] (mg.Kg^{-1})	[Cr TOT] (mg.Kg^{-1})
pH	1.00													
CE (µS. cm^{-1})	-0.28	1.00												
DOC (mg. L^{-1})	0.21	0.00	1.00											
TOC(%)	0.13	0.34	0.07	1.00										
[Cr SPW] (µg.L^{-1})	0.19	0.05	0.06	-0.36	1.00									
[Cu SPW] (µg.L^{-1})	-0.63**	-0.23	-0.14	0.05	-0.49	1.00								
[ZnSPW] (µg.L^{-1})	-0.06	0.00	-0.52	-0.47	0.34	0.10	1.00							
[As SPW] (µg.L^{-1})	0.60*	0.22	0.35	0.21	0.69**	-0.65**	0.02	1.00						
[Zn DGT] (µg.L^{-1})	0.59*	-0.19	0.35	-0.59*	0.58*	-0.62*	0.17	0.50*	1.00					
[Cu DGT] (µg.L^{-1})	0.10	-0.63**	0.31	-0.45	-0.37	0.33	-0.15	-0.44	0.32	1.00				
[As TOT] (mg.Kg^{-1})	-0.59*	0.73**	-0.08	0.26	-0.27	0.41	0.21	-0.20	-0.48	-0.28	1.00			
[Zn TOT] (mg.Kg^{-1})	-0.41	0.55*	-0.35	-0.46	0.45	-0.32	0.37	0.01	0.19	-0.39	0.29	1.00		
[Cu TOT] (mg.Kg^{-1})	-0.61*	-0.23	-0.04	0.06	-0.68**	0.94***	-0.09	-0.77**	-0.60*	0.48	0.41	-0.36	1.00	
[Cr TOT] (mg.Kg^{-1})	0.57*	0.24	0.47	0.07	-0.02	-0.30	0.08	0.43	0.48	0.21	0.21	-0.19	-0.22	1.00

Significance level: ^{NS} Not significant, * P<0.05, **P<0.01, *** P<0.001.

Table 4 . Pearson's correlation coefficients between soil and soil pore water parameters (2011)

2011	pH	CE (µS. cm^{-1})	DOC (mg. L^{-1})	TOC (%)	[Cr SPW] (µg.L^{-1})	[Cu SPW] (µg.L^{-1})	[Zn SPW] (µg.L^{-1})	[As SPW] (µg.L^{-1})	[Zn DGT] (µg.L^{-1})	[Cu DGT] (µg.L^{-1})	[As TOT] (mg.Kg^{-1})	[Zn TOT] (mg.Kg^{-1})	[Cu TOT] (mg.Kg^{-1})	[Cr TOT] (mg.Kg^{-1})
pH	1.00													
CE (µS. cm^{-1})	-0.37 ^{NS}	1.00												
DOC (mg. L^{-1})	0.63**	-0.49 ^{NS}	1.00											
TOC(%)	0.74**	-0.43 ^{NS}	0.94***	1.00										
[Cr SPW] (µg.L^{-1})	0.73**	-0.50*	0.76***	0.89***	1.00									
[Cu SPW] (µg.L^{-1})	-0.07 ^{NS}	0.12 ^{NS}	-0.61*	-0.49 ^{NS}	-0.12 ^{NS}	1.00								
[Zn SPW] (µg.L^{-1})	0.77**	-0.17 ^{NS}	0.53*	0.59*	0.37 ^{NS}	-0.48 ^{NS}	1.00							
[As SPW] (µg.L^{-1})	0.45 ^{NS}	-0.30 ^{NS}	0.87***	0.85***	0.72**	-0.66**	0.43 ^{NS}	1.00						
[Zn DGT] (µg.L^{-1})	0.17 ^{NS}	0.10 ^{NS}	-0.31 ^{NS}	-0.28 ^{NS}	-0.25 ^{NS}	0.35 ^{NS}	0.07 ^{NS}	-0.47 ^{NS}	1.00					
[Cu DGT] (µg.L^{-1})	-0.41^{NS}	0.24 ^{NS}	-0.67**	-0.52*	-0.20 ^{NS}	0.61*	-0.59*	-0.47 ^{NS}	0.17 ^{NS}	1.00				
[As TOT] (mg.Kg^{-1})	-0.60*	0.62*	-0.73***	-0.82***	-0.74***	0.35 ^{NS}	-0.49 ^{NS}	-0.52*	0.14 ^{NS}	0.48 ^{NS}	1.00			
[Zn TOT] (mg.Kg^{-1})	0.02 ^{NS}	0.07 ^{NS}	0.21 ^{NS}	-0.05 ^{NS}	-0.32 ^{NS}	-0.43 ^{NS}	0.32 ^{NS}	0.09 ^{NS}	0.04 ^{NS}	-0.52*	0.29 ^{NS}	1.00		
[Cu TOT] (mg.Kg^{-1})	-0.01 ^{NS}	0.17 ^{NS}	-0.73***	-0.58*	-0.26 ^{NS}	0.93***	-0.29 ^{NS}	-0.77***	0.52*	0.61*	0.41 ^{NS}	-0.36 ^{NS}	1.00	
[Cr TOT] (mg.Kg^{-1})	-0.01 ^{NS}	0.37 ^{NS}	0.11 ^{NS}	0.24 ^{NS}	0.30 ^{NS}	-0.16 ^{NS}	-0.03 ^{NS}	0.51*	-0.38 ^{NS}	0.31 ^{NS}	0.21 ^{NS}	-0.19 ^{NS}	-0.22 ^{NS}	1.00

Significance level: ^{NS} Not significant, * P<0.05, **P<0.01, *** P<0.001.

3.4. Phytoavailability of PTTE

3.4.1. Concentration of PTTE in the bean leaves

Table (5) shows the concentrations of Cu, Cr, Zn and As measured in the leave beans (BL) cultivated in the laboratory as an indicator of the effectiveness of accumulator plants (sun flower, tobacco) to decreases the phytoavailability of PTTE in the contaminated soils. Plants grown on the site significantly decreased the Cu, Cr, Zn and As concentrations in the BL from 2010 to 2011. The average of this decline from 2010 to 2011was about 49% for Cu, 43% for Cr, 70% for Zn and 22% for As.

The table also shows that the increasing concentrations of Cu in the soil led to increasing concentrations of Cu in the beans leaves. The distribution of the total soil Cu concentration values in two cluster (200- 500 and 800– 1200 mg Cu kg^{-1}) resulted in a linear relationship with the Cu concentration in the BL in 2010 and 2011, where these concentrations increased from 40,57±1,32 $\mu g.L^{-1}$ to151,12±1,58 $\mu g.L^{-1}$ (Untreated soil) in 2010 and from 22,85±0,35 $\mu g.L^{-1}$ to 65,50±0,42 $\mu g.L^{-1}$ in 2011. These increased concentrations of Cu in the plots did not show the same effect on the As, Cr and Zn concentrations in the BL.

If we compare the total soil PTTE concentrations in contaminated soil and in the control one (CTRL), we will notice that the concentration of copper in the control soil is less than the others contaminated soils. However the concentration of As, Cr and Zn was relatively higher than the contaminated soils. In contrast the untreated contaminated soil (UNT) presented the highest concentration of Cu in the BL and the lowest concentration of Zn, Cr and As in the BL compared with the others contaminated and control soil (Tab. 5).

Phytotoxic ranges of Cu for most plants are (in mg Cu kg^{-1}), e.g., 15–30 (MacNicol and Beckett, 1985), 25–40 (Chaney 1989), and 10–70 (Gupta and Gupta 1998). This means that the concentrations of Cu which have been measured in our samples exceed the limits identified of previously study especially in the untreated soil which presented the highest concentrations of Cu in 2010 (173,12±1,58) and 2011 (86,15±0,35) compared to the rest of soils.

In the case of Cu, we can notice that there is a strong correlation between the concentrations of Cu in the BL and 1) the total soil concentration of Cu soil (R^2=0,96; R^2=0,99), and with 2) the Cu measured in the SPW (R^2 = 0,86; R^2= 0.94) and 3) with the available fraction of Cu (R^2=0,53; R^2 = 0.58) in 2010 and 2011 respectively. A very strong negative correlation was observed between the Zn in the BL and the increasing total concentration of Cu in the top soils in 2011 (R^2= -0.88). In addition Cr and Zn measured in the BL strongly correlated with DOC and TOC measured in 2011(R^2> 0.65). The correlation was also very weak between the concentrations of others PTTE (Cr, As and Zn) measured in 2010 and 2011 in the BL and the other measured parameters (Tab. 6, 7).

Table 5: Concentrations of Cu, Cr, Zn, As measured the leave beans and their dry weight

	Dry Weight (g)2010	Dry Weight g(2011)	Cu (2010) (mg.Kg^{-1})	Cu(2011) (mg.Kg^{-1})	Zn(2010) (mg.Kg^{-1})	Zn(2011) (mg.Kg^{-1})	Cr(2010) (mg.Kg^{-1})	Cr(2011) (mg.Kg^{-1})	As(2010) (mg.Kg^{-1})	As(2011) (mg.Kg^{-1})
T-20	0,12 ±0,00 ***	0,11±0,01 NS	40,57±1,32 ***	22,85±0,35 ***	72,02±0,54 ***	46,05±0,49 ***	2,83±0,13 ***	0,71±0,12 ***	0,42±0,01 ***	0,20±0,03 NS
T-8	0,05±0,00 ***	0,10±0,00 NS	74,91±0,42 ***	29,05±0,07 ***	77,77±0,90 ***	49,30±0,28 ***	2,60±0,16 ***	1,00±0,01 ***	1,33±0,04 ***	0,16±0,01 NS
T-14	0,21±0,01 ***	0,12±0,01 NS	60,10±0,01 ***	29,90±0,42 ***	56,24±0,80 ***	39,30±0,57 ***	2,17±0,09 ***	0,89±0,09 ***	0,70±0,00 ***	0,19±0,02 NS
T-13	0,15±0,00 ***	0,11±0,01 NS	70,40±0,42 ***	40,35±0,21 ***	46,34±0,51 ***	38,85±0,35 ***	1,63±0,03 ***	1,20±0,08 ***	0,81±0,01 ***	0,25±0,01 NS
T-26	0,09±0,00 ***	0,11±0,02 NS	142,50±2,12 ***	59,95±0,21 ***	68,86±1,20 ***	39,85±0,49 ***	1,29±0,03 ***	0,64±0,02 ***	1,17±0,06 ***	0,20±0,06 NS
T-31(UNT)	0,12±0,01 ***	0,13±0,00 NS	173,12±1,58 ***	86,15±0,35 ***	40,44±0,39 ***	33,35±0,49 ***	1,47±0,05 ***	0,67±0,04 ***	1,34±0,06 ***	0,18±0,01 NS
T-30	0,09±0,00 ***	0,10±0,00 NS	151,12±1,58 ***	65,50±0,42 ***	67,75±1,48 ***	39,15±0,21 ***	3,75±0,09 ***	0,95±0,05 ***	1,13±0,05 ***	0,15±0,01 NS
control	0,09±0,00 ***	0,10±0,00 NS	38,02±1,25 ***	19,50±0,28 ***	83,07±0,56 ***	53,65±0,07 ***	3,39±0,25 ***	1,46±0,09 ***	1,60±0,03 ***	0,23±0,02 NS

Significance level: NS Not significant, * P<0.05, ** P<0.01, *** P<0.001.

Table 6. Pearson's correlation coefficients between PTTE concentrations in the BL and other soil and soil pore water parameters (2010)

2010	[Zn PLT] (mg.Kg^{-1})	[Cr PLT] (mg.Kg^{-1})	[Cu PLT] (mg.Kg^{-1})	[As PLT] (mg.Kg^{-1})
pH	0,56*	0,50*	-0,52*	0,27
CE (µS. cm^{-1})	-0,07	-0,06	-0,34	-0,88***
DOC (mg. L^{-1})	0,18	0,72**	0,00	-0,22
TOC(%)	0,20	-0,10	0,15	-0,26
[Cr SPW] (µg.L^{-1})	0,25	0,22	-0,73**	-0,14
[Cu SPW] (µg.L^{-1})	-0,75**	-0,61*	0,86***	0,10
[Zn SPW] (µg.L^{-1})	-0,54*	-0,52*	-0,31	-0,08
[As SPW] (µg.L^{-1})	0,46	0,45	-0,76**	-0,29
[Zn DGT] (µg.L^{-1})	0,35	0,65**	-0,61*	0,16
[Cu DGT] (µg.L^{-1})	-0,16	0,31	0,53*	0,59*
[Cr PLT] (mg.Kg^{-1})	0,65**	1,00	-0,31	0,12
[Cu PLT] (mg.Kg^{-1})	-0,44	-0,31	1,00	0,33
[Zn PLT] (mg.Kg^{-1})	1,00	0,65**	-0,44	0,26
[As PLT] (mg.Kg^{-1})	0,26	0,12	0,33	1,00
[As TOT] (mg.Kg^{-1})	-0,68**	-0,43	0,21	-0,71**
[Zn TOT] (mg.Kg^{-1})	-0,03	-0,06	-0,46	-0,37
[Cu TOT] (mg.Kg^{-1})	-0,69**	-0,45	0,96***	0,16
[Cr TOT] (mg.Kg^{-1})	-0,08	0,43	-0,32	-0,30

Significance level: NS Not significant, * P<0.05, ** P<0.01, *** P<0.001.

Table 7. Pearson's correlation coefficients between PTTE concentrations in the BL and other soil and soil pore water parameters (2011)

2011	[Zn PLT] (mg.Kg^{-1})	[Cr PLT] (mg.Kg^{-1})	[Cu PLT] (mg.Kg^{-1})	[As PLT] (mg.Kg^{-1})
pH	0,25	0,51*	0,08	0,24
CE (µS. cm^{-1})	-0,60*	-0,26	0,05	0,17
DOC (mg. L^{-1})	0,85***	0,65**	-0,63**	0,33
TOC(%)	0,73**	0,75**	-0,49	0,29
[Cr SPW] (µg.L^{-1})	0,52*	0,65**	-0,14	0,26
[Cu SPW] (µg.L^{-1})	-0,79***	-0,59**	0,94***	-0,16
[Zn SPW] (µg.L^{-1})	0,36	0,56*	-0,25	0,15
[As SPW] (µg.L^{-1})	0,79***	0,83***	-0,71**	0,42
[Zn DGT] (µg.L^{-1})	-0,48	-0,39	0,51**	-0,07
[Cu DGT] (µg.L^{-1})	-0,61*	-0,27	0,58*	-0,28
[Cr PLT] (mg.Kg^{-1})	0,60*	1,00	-0,52*	0,38
[Cu PLT] (mg.Kg^{-1})	-0,80***	-0,52*	1,00	-0,24
[Zn PLT] (mg.Kg^{-1})	1,00	0,60*	-0,80***	0,11
[As PLT] (mg.Kg^{-1})	0,11	0,38	-0,24	1,00
[As TOT] (mg.Kg^{-1})	-0,66**	-0,51*	0,31	0,01
[Zn TOT] (mg.Kg^{-1})	0,24	-0,16	-0,36	0,09
[Cu TOT] (mg.Kg^{-1})	-0,88***	-0,55*	0,99***	-0,23
[Cr TOT] (mg.Kg^{-1})	0,04	0,56*	-0,25	0,31

Significance level: NS Not significant, * P<0.05, ** P<0.01, *** P<0.001.

3.4.2. Mineral masse of PTTE in the leaves beans

Leaves PTTE (Cu, Cr, As, Zn) mineral mass (g ha^{-1}) was computed with leaves PTTE (Cu, Cr, As, Zn) concentrations (mg. kg^{-1}) and the leaves biomass (g DW. pot^{-1}). We can clearly notice in the figure (3) that the mineral mass of all PTTE (Cu, Cr, As, Zn) decreased significantly from 2010 to 2011. We can also observe that the mineral mass of Cu, Cr, As decreased more than for Zn. The percentage of average decrease of the mineral mass of the PTTE was as follows: 49% for Cu, 55% for Cr, 78% for As whereas a lower mineral mass was observed for the Zn as mentioned earlier (34%).

In 2010 , we could notice that the Cu, Cr, Zn, As mineral mass in the BL for the range

200–500 mg Cu kg^{-1} soil distributed similarly, whereas samples T20 and T14 gave mineral masses of Cu, Cr, Zn and As greater than for T8 and T13 for the same PTTE. We also found that for the range 500-1400 mg Cu kg^{-1} Cu and As behaved in the same way, with the plot T31 recording largest concentrations compared to the others samples. In contrast the mineral mass provided by the plots T (26, 30) was almost equal. We could also notice that Zn acted in a manner contrary to Cu and As, with the mineral mass measured in the sample T31 being lower than the others samples. In the case of Cr, we can see that its mineral mass increased with the increasing concentrations of Cu in the range 500-1400 mg Cu kg^{-1}. Figure (3) shows that Cu mineral masses recorded in 2011 increased with the increasing total soil concentration of Cu for the range from 100-1000 mg Cu kg^{-1}. The Cr mineral masses were very scattered. In the same time Zn and As presented almost the same mineral mass in all the plots in 2011.

If we compare the mineral mass measured in the control soil with those measured in the Cu contaminated soil, we can see that the control soil (CTRL) presented the mineral mass of copper much lower than those of other PTTE of the others soils in 2010 and 2011. The result was not the same for the others metals, where the control soil provided mineral mass of Cr, Zn, As higher or equal to what was obtained in the contaminated soils.

Strong correlation was observed between the mineral mass of Cu and the total concentrations of Cu in the top soils and the total dissolved concentration of Cu in the SPW in 2010 and 2011(R^2 >0.9). The mineral mass of Cr showed a good correlation with the total and dissolved Cr concentration in 2011 (R^2 >0.5). In contrast a negative correlation was found between the mineral mass of As mesured in 2010 and the total As concentration of the top soil (R^2= -57) (Tab. 8,9).

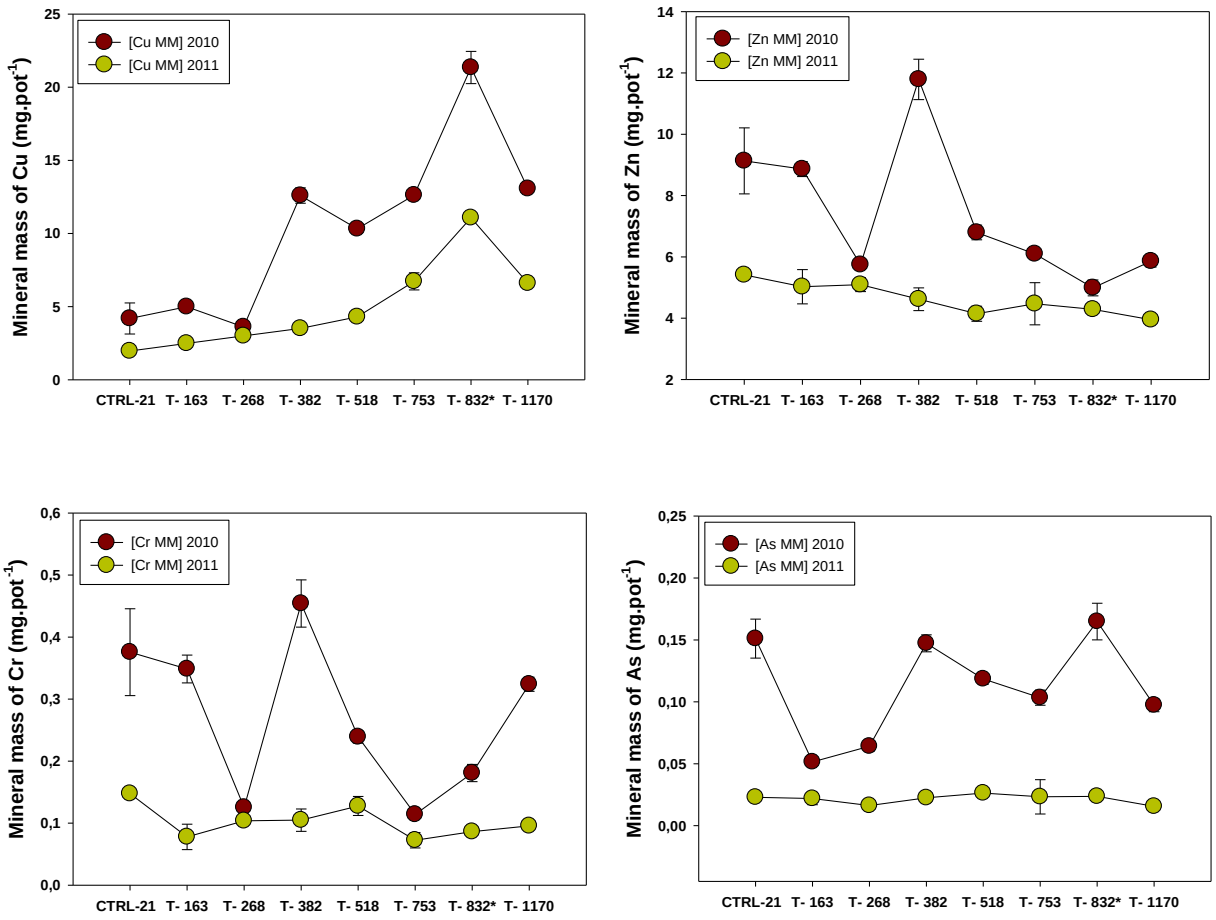

Figure 3: Mineral mass of Cu, Zn, Cr and As measured in the leaves beans.

Table 8: correlation between total soil concentrations, total dissolved SPW concentrations, SPW and soil parameters and planet parameters and the mineral mass of Cu, Cr, Zn and As (2010).

2010	[Cr MM](mg.Pot^{-1})	[Cu MM](mg.Pot^{-1})	[Zn MM](mg.Pot^{-1})	[As MM](mg.Pot^{-1})
pH	0.31	-0.61*	0.26	0.46
CE (µS. cm^{-1})	0.08	-0.27	0.12	-0.42
DOC (mg. L^{-1})	0.68**	0.02	0.38	0.00
TOC(%)	-0.37	-0.17	-0.28	-0.40
[Cr SPW] (µg.L^{-1})	0.44	-0.49	0.47	0.07
[Cu SPW] (µg.L^{-1})	-0.38	0.93***	-0.34	-0.22
[Zn SPW] (µg.L^{-1})	0.06	0.13	0.22	0.07
[As SPW] (µg.L^{-1})	0.50*	-0.70**	0.47	0.09
[Zn DGT] (µg.L^{-1})	0.63	-0.45	0.44	0.41
[Cu DGT] (µg.L^{-1})	0.08	0.47	-0.21	0.29
[Cr PLT] (mg.Kg^{-1})	0.53**	-0.50*	0.17	0.33
[Cu PLT] (mg.Kg^{-1})	-0.51*	0.81***	-0.58*	-0.26
[Zn PLT] (mg.Kg^{-1})	0.10	-0.80***	0.06	0.31
[As PLT] (mg.Kg^{-1})	-0.36	0.06	-0.44	0.42
[Cr MM] (mg.Pot^{-1})	1.00	-0.15	0.88***	0.40
[Cu MM] (mg.Pot^{-1})	-0.15	1.00	-0.14	-0.19
[Zn MM] (mg.Pot^{-1})	0.88***	-0.14	1.00	0.37
[As MM] (mg.Pot^{-1})	0.40	-0.19	0.37	1.00
[As TOT] (mg.Pot^{-1})	-0.16	0.36	-0.16	-0.57*
[Zn TOT] (mg.Kg^{-1})	0.08	-0.29	0.11	-0.05
[Cu TOT] (mg.Kg^{-1})	-0.43	0.92***	-0.48	-0.32
[Cr TOT] (mg.Kg^{-1})	0.49	-0.19	0.28	0.11

Significance level: NS Not significant, * P<0.05, **P<0.01, *** P<0.001.

Table 9: correlation between total soil, solution and planet parameters and the mineral mass of Cu, Cr, Zn and As (2011).

2011	[Cr MM] (mg.Pot^{-1})	[Cu MM] (mg.Pot^{-1})	[Zn MM] (mg.Pot^{-1})	[As MM] (mg.Pot^{-1})
pH	0.54*	0.15	0.28	0.18
CE (µS. cm^{-1})	-0.13	0.08	-0.42	0.28
DOC (mg. L^{-1})	0.57*	-0.60*	0.72**	0.10
TOC(%)	0.67**	-0.48	0.56*	0.05
[Cr SPW] (µg.L^{-1})	0.57*	-0.18	0.32	0.05
[Cu SPW] (µg.L^{-1})	-0.55*	0.90***	-0.69**	0.05
[Zn SPW] (µg.L^{-1})	0.63**	-0.15	0.46	0.12
[As SPW] (µg.L^{-1})	0.73**	-0.72**	0.53*	0.10
[Zn DGT] (µg.L^{-1})	-0.30	0.57*	-0.24	0.13
[Cu DGT] (µg.L^{-1})	-0.32	0.45	-0.77***	-0.24
[Cr PLT] (mg.Kg^{-1})	0.96***	-0.54*	0.36	0.10
[Cu PLT] (mg.Kg^{-1})	-0.45	0.98***	-0.66**	0.00
[Zn PLT] (mg.Kg^{-1})	0.47	-0.80***	0.80***	-0.14
[As PLT] (mg.Kg^{-1})	0.51*	-0.15	0.36	0.92***
[Cr MM] (mg.Pot^{-1})	1.00	-0.42	0.41	0.30
[Cu MM] (mg.Pot^{-1})	-0.42	1.00	-0.56*	0.13
[Zn MM] (mg.Pot^{-1})	0.41	-0.56*	1.00	0.30
[As MM] (mg.Pot^{-1})	0.30	0.13	0.30	1.00
[As TOT] (mg.Kg^{-1})	-0.44	0.31	-0.54*	0.13
[Zn TOT] (mg.Kg^{-1})	-0.10	-0.24	0.45	0.13
[Cu TOT] (mg.Kg^{-1})	-0.47	0.97***	-0.72**	0.02
[Cr TOT] (mg.Kg^{-1})	0.50*	-0.33	-0.25	0.06

Significance level: NS Not significant, * P<0.05, **P<0.01, *** P<0.001

4. Conclusion

Changes in the mobility, soil exposure intensity and phytoavailability of Cu, Zn, Cr and As were investigated at a wood preservation site, in topsoils of field plots amended with dolomitic limestone (DL) and compost (OM) and phytomanaged with a crop rotation of sunflower and tobbaco, after two years. Sub-plots with the presence of increasing concentrations of Cu in their soils but identical physico-chemical parameters were used together with a control uncontaminated plot.

Cu total dissolved concentrations measured in the SPW of the contaminated soil and the control soil (CTRL) was much higher than the other PTTE (Zn, As, Cr) measured in 2010 and 2011. The crop rotation of sunflower and tobacco significantly decreased the total dissolved concentrations of Cu, Zn , Cr and As in the SPW. However we did not notice a significant difference between the total dissolved concentrations of Zn, Cr and As in the SPW of the control soil (CTRL) and the contaminated soil samples. The available concentrations for all PTTE have declined significantly after two years of crop rotation. The soil exposure intensity as defined by the available concentrations of the PTTE also increased for Cu with the total Cu soil concentration for the contaminated soils. The phytoavailability of Cu as studied by the BL concentratipns or the mineral mass of PTT increased with the total soil concentrations. The phytoavailability of the PTTE also decreased as the foliar PTTE concentrations together with the mineral masses.

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Chapitre 2.3

Phytostabilisation assistée d'un techno-sol contaminé par une forte concentration d'éléments traces, par des amendements organiques frais et matures

2.3.1. Résumé de la publication

Dans les articles précédents nos travaux ont été appliqués sur un site naturel contaminé en sulfate de Cu et en sels du Cu (CCA) utilisés pour traiter le bois.

Dans cet article notre travail a porté sur un techno-sol développé sur une décharge de co-produits métallurgiques appartenant à Industeel-Loire (groupe ArcelorMittal) située sur la commune de Châteauneuf (Loire) présentant une multi-contamination métallique.

L'objectif de notre travail sur ce site a été d'évaluer la capacité de la phytostabilation assistée par deux différents amendements organiques (boues d'épuration compostées (CSS) et bois raméal fragmenté (RCW)) à diminuer la mobilité, la phytodisponibilité et l'intensité de l'exposition du sol de plusieurs PTTE tels que le molybdène (Mo), le chrome (Cr), le zinc (Zn), le cuivre (Cu), le cobalt (Co) et l'arsenic (As) sur un techno-sol.

Les travaux ont porté sur neuf parcelles traitées par phytostabilisation avec des graminées et avec différents types d'amendements. Les neuf parcelles sont: (A) 3 parcelles de sols non amendés (NE), (B) 3 parcelles de sol amendés avec des matières organiques fraiches, le bois raméal fragmenté (RCW) et (C) 3 parcelles de sols traités avec des boues d'épuration compostées (CSS) ayant une grande maturité. Sur les trois premières parcelles (des groupes A, B et C) un mélange de plantes classiques a été semé; sur les trois parcelles suivantes, des plantes métallicoles ont été cultivées et en fin des plantes non-cultivées se sont développées sur les trois dernières parcelles.

Pour évaluer les performances des techniques de phytostabilisation assistée, les sols des 9 parcelles ont été échantillonnés, mélangés, séchés à l'air et tamisé (<2 mm, filet de nylon) et combinées pour produire un échantillon de sol composite (1 kg FW). Ensuite ces sols ont été analysés en laboratoire. Les concentrations totales des PTTE dissous ont été déterminées dans l'eau interstitielle du sol (SPW) échantillonnée par des échantillonneurs Rhizon, placés dans le sol 24h après avoir monté l'humidité du sol à 80% (capacité au champ) pendant une semaine. L'intensité de l'exposition du sol a été évaluée par des sondes DGT (Chelex 100) (gradient de diffusion dans les couches minces), qui a été mis dans le sol manuellement quand il avait 80% d'humidité également. Le phytodisponibilité des PTTE a été caractérisée par des tests de germination avec des haricots nains pour lesquels les concentrations foliaires et les minéralomasses en PTTE ont été déterminées. Toutes les concentrations des PTTE dans les SPW extraits par les DGT et dans les feuilles de haricot ont été déterminées par ICP-MS (Element 2, Thermofischer).

Les résultats de la présente étude indiquent que l'ajout de RCW frais et de CSS mature a un effet positif sur les techno-sols fortement contaminés en PTTE. On a trouvé que le RCW a diminué la mobilité de tous les éléments étudiés dans la solution du sol (SPW), tandis que le CSS réduit la mobilité de Mo, Cr et Co, alors qu'il augmente la mobilité de Zn, Cu en comparaison avec le sol sans amendements. L'intensité de l'exposition du sol au Zn évalué par la DGT n'était pas significativement modifiée par l'ajout de RCW et CSS, tandis que l'intensité de l'exposition au Cr évaluée par la DGT a été significativement diminuée après l'addition RCW par rapport au sol traité avec CSS et le sol sans amendement. En comparaison, Cu et Co étaient non labiles dans les trois techno-sols. Les deux amendements RCW et CSS diminuent la concentration foliaire de Mo, Zn, Cr, As et Co dans les feuilles d'haricots, alors que les deux amendements augmentent la concentration foliaire de Cu. De plus l'amendement RCW a été plus efficace que CSS pour réduire la minéralomasse de Mo, Cr et Co dans les feuilles de haricots.

Finalement nous avons trouvé que la phytostabilisation assistée par les deux amendements organiques a été efficace pour stabiliser les PTTE dans les techno-sols, spécialement dans le cas du RCW, mais il faudra refaire des expériences pour vérifier ces résultats et voir l'effet du RCW avec le temps.

2.3.2. Article 3 (en préparation)

Phytostabilization of techno-soils contaminated with high concentration of trace elements assisted by fresh and mature organic amendments

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Abstract: Organic compounds resulting from the decomposition of organic amendments used in the remediation of trace elements (TE) contaminated soils change the soil physical and chemical properties shortly after their addition and alter the speciation of TE. The mobility, phytoavailability and soil exposure intensity of molybdenum (Mo), chromium (Cr), zinc (Zn), cooper (Cu), Cobalt (Co) and Arsenic (As) was evaluated in the phytoremediation of contaminated techno-soils after the addition of two organic matter types, fresh ramial chipped wood (RCW) and composted sewage sludge (CSS). The experiment consisted of nine main treatment blocks: (A) 3X unamended soil (NE), (B) 3X soil amended with RCW and (C) 3X soil amended with CSS having a high maturity. Total dissolved TE concentrations were determined in soil pore water (SPW) sampled by Rhizon samplers. The soil exposure intensity was assessed by standard Chelex 100 DGT (diffusive gradient in thin films) probes. The TE phytoavailability was characterized by growing dwarf beans on potted soils and analyzing their foliar TE concentrations.

The results of the present study indicate that the addition of fresh RCW and CSS has a positive effect contaminated techno-soils. RCW decreased the mobility of all studied TE in the SPW, whereas CSS reduced the mobility of Mo, Cr and Co, while it increased the mobility of Zn, Cu and As compared with the NE soil. The Zn soil exposure intensity assessed by DGT were not significantly changed by the addition of RCW and CSS, while the Cr soil exposure intensity assessed by DGT were significantly decreased after RCW addition compared with the soil treated with CSS and the NE soil. In contrast Cu and Co were non labile in the three soils. Both RCW and CSS decreased the foliar concentration of Mo, Zn, Cr, As and Co in the beans leaves whereas the two amendments increased the foliar Cu concentration. Soil amended with RCW was more efficient than CSS to reduce the mineral mass of Mo, Cr and Co in the beans leaves.

Key words: Soil contamination, phytostabilisation, Trace elements, Organic amendment, Mobility, phytovailability, soil exposure intensity

1. Introduction

Among anthropo-soils, technosoils and hortic antropo-soils are considered to be the most exposed to pollution (Bulgariu et al., 2007; Thornton, 1991: Florea and Munteanu, 2003, IUSS, 2006). The high concentration, distribution and migration of TE in these soils represent an important environmental problem due to the high toxicity of these elements that may cause major perturbations to soil ecosystems (Alloway, 1995; Kabata-Pendias and Pendias, 1992; Ross, 1994). In fact the contamination of anthropo-soils with high levels of TE is more problematic than for other soils because most properties of these types of soils may change with time, making their handling more difficult (Kelly et al., 1996). Therefore the contamination of an anthropo-soil with TE requires special soil-management techniques to limit the solubility and the bioavailability of metal(oid)s.

Several treatment methods can be used to remediate TE contaminated soils. These techniques include physical and chemical remediation, and agro-ecological engineering methods such as phytoremediation (Chen et al. 1999; Chen et al., 2000). Conventional soil reclamation technologies such as 'dig and dump', soil washing, and sieving are effective but destructive thus not sustainable in terms of consumption of raw materials and waste production(Basta et al., 2004; Raicevic et al., 2005; Dermont et al., 2008). In addition, they are very expensive, particularly for large contaminated sites. Less invasive, low-cost phytotechnologies such as phytostabilization, singly and in combination with *in situ* stabilization (*i.e.* assisted phytostabilization), are potential management options to restore the physical, chemical and biological properties of TE contaminated soils (Mench et al., 2000; Bolan et al., 2003; Pérez de Mora et al., 2005; Raicevic et al., 2005; Kumpiene et al., 2006; 2008). Several mineral and organic amendments such as lime, coal fly ashes, phosphates, red muds, compost, biosolids, iron grit and Fe/Mn/Al oxides can improve phytostabilization and production of plant-based feedstock through the decrease of the solubility, leaching and bioavailability of TE (Lombi et al., 2002; Bolan et al., 2003; Brown et al., 2004; Geebelen et al., 2003; Basta et al., 2004; Kumpiene et al. 2008; Mench et al, 2010). The immobilising effect of such amendments are thought to act through various complex processes *e.g.* adsorption onto mineral surfaces, formation of stable compounds with organic ligands, surface precipitation and ion exchange (Kumpiene et al., 2008, Ahmad et al., 2011). However, these processes are still not well understood and the choice of a particular amendment is often problematic. Thus, cases studies assessing the mobility and bioavailability of TE in contaminated soils managed by assisted phytostabilization are needed to better define the pros and cons of such management options (Mench et al., 2010).

Among the various compounds that can be used in aided phytostabilization, the addition of organic matter amendments such as compost, manure or various organic wastes presents a special interest.

Organic matter additions to soil have long been considered important in maintaining the quality of both natural and managed soils, principally because of their capacity in providing the nutrients to soil's living organisms and through their influence on the soil physical properties. This influence on soils properties depending on several factors such as: the quantity, type and maturation of these organic matters applied to the soil and soil properties respectively. Organic amendments may indeed contain a high proportion of humified organic matter that has a large capacity to immobilize TE through the formation of more or less stable complexes. The formation of these insoluble metal compounds reduces their mobility through the soil profile and the pool available for biota (Geebelen et al., 2003). Moreover organic amendments may enhance the soil fertility and microbial activity, leading to the amelioration of the soil quality in its whole. These overall modifications generally decrease the mobility and the bioavailability of TE, even though temporarily and thus promote the reestablishment of vegetation and increase the plants growth (Castaldi and Melis, 2004, Madej et al., 2006; Branzini and Zubillaga, 2012).

Nevertheless, the effect of organic amendments on metal(loid)s bioavailability depends

on the nature of the organic matter itself, its microbial degradability, the salt content and effects on soil pH and redox potential, as well as on the particular soil type and metal(loid)s concerned (Walker et al. 2003, 2004). However very few comparative studies have been performed so far and the choice of a particular organic amendment in assisted phytostabilization strategies often remain empirical.

The main thrust of this work was to assess the effect of two different organic amendments (composted sewage sludge (CSS) and fresh ramial chipped wood (RCW)) on the mobility, phytoavailability and soil exposure intensity of several TE in a metallurgical technosoil remediated by assisted phytostabilization.

2. Materials and methods

2.1. Site description and experimental design

The studied site is a metallurgical landfill (Industeel-Loire; 45°32' N; 4°38'E) near Lyon, France. It is located behind a steel and iron factory which is still in activity. The site has been used from about 1850 to 2001 to dispose of foundry wastes, slags, fire-bricks and various by-products from the industrial process. It extends over nearly 15 ha for a thickness of 8 to 10 m. The soil ("foundry technosoil" type) is highly contaminated with iron (Fe) and different metals used in various alloys such as aluminum (Al), chromium (Cr), copper (Cu), manganese (Mn), molybdenum (Mo), nickel (Ni), lead (Pb) and zinc (Zn). This site is part of a French network of contaminated sites ("SAFIR" network, http://www.safir-network.com) and is partly dedicated to researches on contaminant transfer, biological impact of contamination and remediation technologies.

The efficiency of organic amendments to decrease TE availability in assisted phytostabilisation was studied on nine $50m^2$ (5 m X 10 m) experimental plots. Plots were prepared in December 2009 by a shallow ploughing (10 to 15cm depth) and were enriched using the following organicamendments: (1) RCW applied at approx. 500 m^3/ha (3 plots), (2) CSS applied at approx. 120 t DM/ha (3 plots) and (3) no organic enrichment (NE) (3 plots). Plots were sown with a mixture of grasses and dicotyledonous herbaceous species in March 2010, *i.e* four months after the plots preparation. At the same time an extensive pedo-geochemical characterization of the different plots was performed (Lespagnol and Bouchardon, 2011). The main pedological characteristics of the soils studied (taken at 0-20 cm depth) are given in table 1. Their total metal concentrations are given in table 2. The average concentration values of metals measured in the soil were 5837 $mg.kg^{-1}$ Cr, 620 $mg.kg^{-1}$ Mo, 1464 $mg.kg^{-1}$ Zn, 444 $mg.kg^{-1}$ Cu and 80 $mg.kg^{-1}$ As (Table 2). These TE concentrations were largely above the limits of TE concentrations measured in the natural unpolluted French soils (Baize, 2000), which confirms the existence of a very important polymetallic contamination.

2.2.Assessment of TE (phyto) availability

TE (phyto) availability in the studied soils was assessed using three different approaches: *i/* the measurement of growth inhibition and TE accumulation in *Phaseolus vulgaris*, *ii*/ the measurement of metal concentration in the soil pore water and *iii*/ the diffusive gradient in thin gel (DGT) method to assess the TE soil exposure intensity.

Table 3: Main pedological characteristics of the soils studied determined four months after the plots preparation. For each treatment, data are the mean of 3 measurements.

Parameters	Treatments		
	RCW	**CSS**	**NE**
Texture	sandy	sandy	sandy
pH	10,63±0.23	10,63±0.12	11,20±0.17
C org (g.kg^{-1})	22.07±5.64	42.30±9.17	19.92±3.00
N (g.kg^{-1})	0,47±0.12	2,33±0.32	0,33±0.06
C/N	47,70±5.10	17,97±0.25	60,00±5.96
CEC(mEq/100 g)	5,63±1.10	6,77±0.15	5,77±0.55
P_2O5 (g.kg^{-1})	0,03±0.00	0,25±0.04	0,02±0.00
K_2O (g.kg^{-1})	0,29±0.05	0,59±0.07	0,09±0.07
CaO (g.kg^{-1})	18,88±6.71	20,70±1.13	24,67±2.17
MgO (g.kg^{-1})	2,66±0.44	1,29±0.09	2,79±0.44
Na_2O (g.kg^{-1})	0,08±0.07	0,14±0.02	0,14±0.03

Table 4: Total TE concentrations (hydrofluoric acid extraction) of the studied soils determined four months after plots preparation. For each treatment, data are the mean of 30 measurements (10 samples by plot, 3 plots by treatment).

Metals	Treatments		
	RCW	**CSS**	**NE**
Cr (mg.kg^{-1})	5397±962	5138±1057	6286±957
Mo (mg.kg^{-1})	545±175	654±62	660±141
Cu (mg.kg^{-1})	418±113	401 ±56	416±22
Zn (mg.kg^{-1})	1314±577	1102±468	1218±647
As (mg.kg^{-1})	80±19	74±11	91±14

2.2.1 Growth inhibition and TE accumulation in *Phaseolus vulgaris*

Soils were taken in May 2011, *i.e* 18 month after ploughing and soil enrichment with RCW and CSS amendments. Each soil sample (approx. 3.0 kg) was made of six independent sub-samples (approx. 0.5 kg) randomly taken from the surface soil (0 – 25 cm) at each of the nine experimental plots. The nine samples (3 x RCW, 3 x CSS and 3 x NE) were sieved at 2 mm. One kilo of each soil was then placed in 1.5 L pots (15 cm D X 14 cm L), and watered up to 80% of the water holding capacity (WHC) with addition of deionized water. Four seeds of dwarf bean (*Phaseolus vulgaris* L. cv. Contender) were then sown in each pot and cultivated under controlled conditions (16 h light/8 h darkness, 25°C/21°C, 150 µmol m^{-2} s^{-1}, 65% relative humidity (ISO 2005). Pots were weighted and watered each day to maintain soil moisture at 80% WHC. After 18 days of culture, plants were harvested, and the dry weight (DW) of all the primary leaves was determined after drying for 24 h at 70°C. In addition, aliquots (35-150 mg) of dried primary bean leaves (BL) were taken for metal analyses and mineral biomass determinations. For digestion, BL samples were placed into 50 ml screw cap Savillex PTFE tubes containing 4 mL 32% HNO_3. Tubes were kept open and heated at 65°C for 2 hours. Caps were then closed and tubes were left overnight at 65°C (12-14h). After this pre-digestion step, 0.5 mL 30% H_2O_2 was added and

tubes were left open at 75°C for 3 hours. Then, 1.5+/-0.5 mL 48% *hydrofluoric acid* was added; tubes were closed and left at 100°C overnight. Finally, tubes were opened and kept at 120°C for 4-5 hours, until complete evaporation. Metals were then solubilised using 6 mL 11 % HNO_3 + 0.1mL H_2O_2, the solutions were gently warmed and made up to 50 mL with deionised water**.** Digests were kept at 4°C until metal analyses.

2.2.2. Extraction and characterization of SPW

Soil moisture samplers (model MOM, Rhizosphere Research Products, Wageningen, the Netherlands), here after called rhizons, were used to extract the SPW from each pot. Rhizons were cleaned with 5% HNO_3 and carefully washed with *deionized water* before use. Three rhizons were placed at 45° in each pot during pots filling. After 15 days of bean culture, rhizons from each pot were let under vacuum for 24 hours (Cattani, 2006) to collect the soil pore solution (≈ 30 mL by pot). Three aliquots (3 ml) of the soil solution from each pot were acidified with HNO_3 (final concentration: 0.1 $mol.L^{-1}$) for metal analyses. Additionally, three aliquots (1.5 ml) were kept untreated for measurements of dissolved organic carbon (DOC). The SPW solutions were stored at 4C° until analyses.

2.2.3. DGT measurements

The potentially available TE concentrations (C_{DGT}) or soil exposure intensity was determined using standard Chelex-100 cylindrical DGT units (DGT Research, Lancaster, UK) with an active surface area of 3.14 cm^2. These DGT devices (Zhang, 2001) consisted of a Chelex 100 binding resin layer (x mm thick) and a polyacrylamide diffusion gel layer (0.8 mm thick), covered with a 0.45 mm filter membrane (0.14 mm thick).

Two DGT probes were manually inserted into the humid topsoil (80% RCW) of each pot, just after the bean harvest. After a 24 h period, metals accumulated in the resin-gel layer were extracted by immersion for 24h into 1mL of 5% HNO_3. This solution was further diluted 10 times before TE analysis.

2.3. Measurements of TE in bean extracts, SPW and DGT probes

TE concentrations were determined by HR-ICP-MS (Element 2, Thermo Fischer, Waltham, USA) using a Twinnabar cyclonic spray chamber and a Micromist nebulizer (Glass Expansion, Melbourne, Australia). The accuracy and precision of analyses was checked by performing calibrations with a standard reference water solution (1000 ppm, Fisher scientific).

For the primary BL digests, six TE (As, Co, Cr, Cu, Mo and Zn) were analyzed. Three repeated measurements were performed for each digest, generating 27 testing results (3 replicates x 9 digests). Total TE concentrations in leaves were first determined on a mg TE/g DW (dry weight) basis. In addition, the mineral masses (expressed in mg TE/pot) were calculated by multiplying the element concentration in leaves by the total DW of the harvested material.

For the SPW solutions extracted with Rhizon samplers, the same TE (As, Co, Cr, Cu, Mo and Zn) were analyzed. Results were expressed on a µg TE/L soil solution basis.

Lastly, for the TE extracted with DGT probes, only Co, Cu, Cr and Zn concentrations were determined because of the selectivity of the chelex 100 resin. The total mass of metal (M) accumulated per unit area of the DGT probe over its deployment time (t) was calculated as follow:

$$M = C_e (V_{HNO3} + V_{gel})/fe$$

where C_e is the concentration of metals in the 1M HNO_3 elution solution (in µg/L), V_{HNO3} is the volume of HNO_3 used to extract metals from the resin gel, V_{gel} is the volume of the resin gel, typically 0.15 ml, and *fe* is the elution factor for each metal, typically 0.8.

The potentially available trace elements concentrations (C_{DGT}) were then calculated using the following equation:

$$C_{DGT} = M\Delta g/(D\ t\ A)$$

where Δg is the thickness of the diffusive gel plus the thickness of the filter membrane, D is the diffusion coefficient of metal in the gel, t is deployment time and A is the exposure area.

Finally, the extent of metal depletion of soil pore water at the DGT interface (R) was calculated by the ratio:

$$R = C_{DGT}/C_{SPW}$$

where C_{SPW} is metal concentrations in the soil pore water, determined by rhizon samplers.

2.4. Measurements of total and dissolved organic carbon

Total organic carbon (TOC) in the soils studied was determined using a Rock-Eval 6 apparatus (Vinci Technologies) (Espitalié et al. (1985a, b) and Lafargue et al. (1998)). This instrument uses a ramped temperature pyrolysis technique, where a small amount of material (70 - 100 mg) is heated in an inert atmosphere (helium or nitrogen) and combusted with air, to measure several key geochemical parameters, including the total organic carbon (TOC). Measurements of TOC were performed in samples taken from the potted soils, just before bean sowing, and after drying the soil at 105C° and milled it very finely to obtain homogeneous samples.

Dissolved organic carbon (DOC) was measured in SPW collected with Rhizon samplers. Analyses were performed with a Shimadzu© TOC 5000A carbon analyzer. The accuracy of the instrument was checked by performing calibration with a standard reference solution of potassium hydrogen phthalate (KHP) at a concentration of 1000 mg Carbon/L. Four repeated measurements of DOC were performed for each SPW solution.

2.5. Other measurements

The pH and electric conductivity (EC) of the studied soils were measured at the beginning of the experiment, before bean sowing. Measures were performed in deionized water with a soil: water ratio of 1: 2.5 (S: W) (NF ISO 10390).

pH and EC were also determined in the SPW solutions taken with Rhizon samplers. Additionally, the concentrations of major cations (Na^+, K^+, Ca^{2+}, and NH_4^+) and anions (NO_3^-, SO_4^{2-}, and Cl^-) were determined by ionic chromatography (Dionex ICS-2000, Sunnyvale, CA), using a CS16A column for cations and a AS17 column for anions.

2.6. Statistical analyses

One way analysis of variance (ANOVA), followed by Tukey post hoc comparisons were performed on the total dissolved SPW concentrations, DGT concentrations, R ratios, foliar element concentrations, foliar mineral masses and primary bean leaves DW yields to evaluate the treatment influence on TE (phyto)availability. Pearson correlation coefficients (linear regression) between soil, SPW and plant parameters were also calculated. Differences were considered statistically significant at $p < 0.05$. All statistical analyses were performed using the Statistica V. 6 software (StatSoft).

3. Results

3.1. SPW characteristics

Table (3) shows the physico-chemical characteristics of the soil (i.e. pH, EC and TOC) and SPW (pH, DOC, EC and the concentrations of major cations and anions) determined for the treated (RCW and CSS) and the untreated subplots (NE) one year after the addition of amendments. The table presents the average values and standard deviations for the nine plots (3 replicates X 3 types of soil). The experiments reveal that the additions of the amendments (RCW and CSS) in the soils have several effects.

First the soil pH did not change significantly between the treated and untreated soils ($P > 0.05$), it slightly decreased by 3% in the soil treated with RCW and CSS compared to the untreated soil (NE). Similar results were found by Vaca et al, (2011)

who investigated the effect of CSS on the soil proprieties and TE mobility. These authors found that the CSS did not have a significant effect on the soil pH and that the addition of CSS to the soil also decreased the soil pH by 3% compared with the control soil. Similar results were found by Soumare et al., 2004 who studied the effect of RCW and the litter compost (LC) on the contaminated soil properties compared with a control soil; they found that RCW decreased slightly the soil pH by 3% compared with the control soil. Boisch, (1997) tested the effect of biowaste compost (BWC) on the soil properties; they found that this amendment increased the soil salinity, but they did not see any effect of soil amendment on the soil organic matter (SOM) content, pH and EC.

The values of the DOC decreased by 2 (52%) and 4 (76%) fold, the TOC by 2 (55%) and 3.5 (72%) fold and the EC by 1.8 (44 %) and 1.3 (26%) fold for the RCW and CSS respectively compared with the untreated soil (NE). Vaca et al., (2011) also found that the addition of CSS to the soil increased its SOM content by 2 fold compared to the soil without amendments, whereas Soumare et al., 2004 , found that the application of RCW to the soil increased the quantity of SOM in the soil by 2 fold compared with the control soil. Additionnaly Bragato et al.,1998 investigated two types of organic amendment (dehydrated Sludge and sewage sludge compost (SSC)) on a salty loam soil, they found that the application of a dehydrated Sludge and SSC- compost did increase the soil TOC-content (from 0,71 to 0,86 %; i.e. a relative increase of 21 %) compared with the control soil. Additionally soil amendments had an impact on the cations and anions concentration in the SPW. We can note that the addition of the RCW and CSS decreased the anions and some cations concentration such as (Cl^- , $NO3^-$, $SO4^{2-}$, $PO4^{3-}$; Na^+, Ca^{+2}), while it increased the concentration of other cations such as (Mg^{2+}, Fe^{2+}, Mn^{2+}) in comparison with the untreated soil.

Table 5: Physico-chemical characteristics of the soil and SPW (mean values+ standard deviations) (n=9)

		RCW	CSS	NE
Soil variables	**pH (Soil)**	9.08±0.07	9.07±0.12	9.34±0.18
	CE (Soil)(µS.cm^{-1})	566.67±49.4	428.00±15.71	317.33±54.86
	TOC(Soil)(%)	1.97±0.34	3.14±0.34	0.89±0.24
SPWvariables	**pH**	8.91±0.04	8.71±0.11	8.53±0.27
	CE (µS.cm^{-1})	1750.33±148.75	1786.66±174.17	1474,66± 142.71
	DOC (mg.l^{-1})	29.27±1.79	59.93±14.14	14.09±1.97
	Cl^- (mg.l^{-1})	44.98±28.32	47.45±6.12	64.89±0.49
	NO_3^- (mg.l^{-1})	ND	185.27±24.91	115.36±58.43
	SO_4^{-2} (mg.l^{-1})	94.43±50.48	183.71±77.39	358.01±27.00
	PO_4^{-3} (mg.l^{-1})	8.43±5.50	6.62±0.63	10.16±1.01
	Na^+ (mg.l^{-1})	28.21±4.80	26.56±3.73	33.07±20.57
	Mg^{+2} (mg.l^{-1})	309.26±31.60	234.54±24.73	127.99±86.71
	K^+ (mg.l^{-1})	13.47±2.49	12.91±0.63	12.11±7.55
	Ca^{+2} (mg.l^{-1})	16.49±7.79	57.06±12.13	19.86±10.48

3.2. SPW concentrations of TE

The most obvious feature of the studied subplots was its high level of contamination in the soil pore water by various TE such as Mo, Zn, Cu, Cr, As and Co (Fig. 2). The average concentration of TE collected from the 9 SPW extracted from the 9 potted contaminated soils showed a high effect of soil amendment on the TE concentrations in the

SPW. These concentrations are often related to the total TE concentration in the soil. The average of their concentrations in the treated and untreated contaminated soils (RCW, CSS and NE) were as follows: 3.32 $mg.l^{-1}$ of MO, 2.7.7 $\mu g.l^{-1}$ of Zn , 41.1 $\mu g.l^{-1}$ of Cr ,43.0 $\mu g.l^{-1}$ of Cu,15.5 $\mu g.l^{-1}$ of As, and 2.6 $\mu g.l^{-1}$ of Co (Fig. 2). The values presented by figure 2 are the median, 25%-75% and the Min-Max for (n=9).

Organic amendments (RCW and CSS) affected significantly the [Mo_{SPW}] of the treated soils compared with untreated soil (P=0.02). RCW added to the soil decreased slightly [Mo_{SPW}] in the soil by (0.4%), whereas this decrease was more significant in the soil treated with the CSS (36%) compared with the untreated soil (NE). Our results demonstrated that CSS was the best amendment to reduce [Mo_{SPW}] in the contaminated soils (fig.2.a).

Figure (2, b) shows that soils amended with organic amendments had two different significant effects on the mobility of Zn in the soil. First we can observe that the addition of RCW had the capacity to reduce the Zn SPW concentration compared with the untreated soil (NE). This decrease in [Zn_{spw}] from the soil (NE) was about 57% (from 172.94±40.85 ($\mu g.l^{-1}$) in the soil (NE) to 72.79±27.31 ($\mu g.l^{-1}$) in the soil treated with RCW). In contrast the CSS had an inverse effect on the Zn mobility, where the addition of CSS to the soil increased the concentration of mobile Zn compared to the untreated soil by 54% (from 172.94±40.85 ($\mu g.l^{-1}$) in the soil (NE) to 377.40±33.78 ($\mu g.l^{-1}$) in the soil treated with (CSS)). A very significant correlation (r= 0.76, r= 0.71) was observed between each of the dissolved organic matter (DOC) and total organic matter (TOC) and the SPW zinc concentration in the soil solution [Zn_{spw}] respectively.

Figure (2, c) shows the effect of organic amendments on the SPW Cu concentration. Organic amendments had an significant effect on the mobility of Cu in the soil (P=0.000001), where RCW has the capacity to reduce [Cu_{spw}] by 4 fold compared with the untreated soil (NE) (e.g. decreased from 18.05±0.89 ($\mu g.\ l^{-1}$) in the soil (NE), to 4.59±0.86 ($\mu g.\ l^{-1}$)) in the soil treated with RCW. In contrast CSS have a completely different effect, where it increased the mobile Cu concentration by 6 fold compared with the soil without amendment (e.g. increased from 18.05±0.89 ($\mu g.\ L^{-1}$) in the soil (NE), to 106.45±21.15 ($\mu g.\ l^{-1}$) in the soil treated with (CSS)). Additionnaly we found that total Cu concentration measured in the SPW [Cu_{spw}] was highly related with the total organic matter content of the soils ([Cu_{SPW}] ($\mu g.l^{-1}$) = -48.72 + 43.669 * TOC (%), Correlation: r = 0.8321), and dissolved organic matter of the SPW ([Cu_{SPW}]($\mu g.\ l^{-1}$) = -39.57 + 2.2615 * DOC ($mg.l^{-1}$), Correlation: r = 0.91562).

Effect of organic amendments (CSS and RCW) on the mobility of Cr in the soil can be clearly observed in figure (2, d). We can notice that organic amendments significantly reduced the concentrations of SPW chromium in the treated soil by nearly 1.7 fold compared with the untreated soil (p= 0.00006). This reduction was by 39% for the soil amended with RCW (e.g. from 56.02±9.86 ($\mu g.l^{-1}$) in the soil NE to 34.97±7.59 ($\mu g.l^{-1}$) in the RCW soil) whereas the CSS reduced the [Cr_{spw}] by 42% compared with NE soils (e.g. from 56.02±9.86 ($\mu g.l^{-1}$) in the soil NE to 32.48±8.27 ($\mu g.l^{-1}$) in the CSS soil). A good correlation was observed between the soil pH and the [Cr_{spw}] (r=0.68), whereas DOC and TOC values were significantly negatively correlated with tthe Cr SPW concentration (r = -0.716. p=<0.05 and r=-0.714, p=<0.05 respectively).

Figure (2, e) shows the different effects of organic amendments on the As SPW concentrations. The addition of RCW to the soil did not have any effect on the As SPW concentration compared with the untreated soil (11.63±3.96 ($\mu g.l^{-1}$) of [As_{spw}] in the untreated soil and 11.72±3.41 ($\mu g.l^{-1}$) of [As_{spw}] in the soil treated with RCW. In contrast we noticed a significant effect of the CSS on the SPW concentration of As compared to the soil treated with RCW and NE soils (p=0.000004). Soil amended with CSS increased the As mobility by 49.4% compared with the RCW soil and 50.2% compared with the NE soil (e.g. As concentration increased from (11.63±3.96 ($\mu g.l^{-1}$) of [As_{spw}] in the NE soil to 23.32±4.51 ($\mu g.l^{-1}$) of [As_{spw}] in the soil treated with CSS. A very strong correlation was observed between DOC and TOC measured in SPW

and in soil and the [As_{spw}] concentration measured in the SPW solutions; this correlation was r = 0.82, p=<0.05 and r=0.80, p<0.05 respectively.

RCW and CSS added to the soil significantly effected on the Co SPW concentrations in the treated soil and untreated soil (p= $1.64E^{-11}$). Effect of RCW and CSS added to the soil on the mobility of Co in the soil were similar to their impact on the mobility of Cu. Figure (2, f) shows that the addition of RCW to the contaminated soil reduced [Co_{spw}] by 48% compared with the untreated soil NE (e.g. from 1.32±0.48 (µg.l^{-1}) in the (NE) soil to 0.68±0.3248 (µg.l^{-1}) in the (RCW) soil). In constrat the addition of CSS had an impact completely different from that of RCW on the Co mobility. CSS increased the Co mobility by 82% compared to the (NE) soil (e.g. from 1.32±0.48 (µg.l^{-1}) in the (NE) soil to 7.17±1.36 (µg.l^{-1}) in the (CSS) soil. The SPW Co concentration shows very good correlation with each of DOC and TOC, this relation was very strong with DOC values (r = 0.89, p=<0.01), whereas it was less strong with the TOC(r = 0.79, p=<0.05).

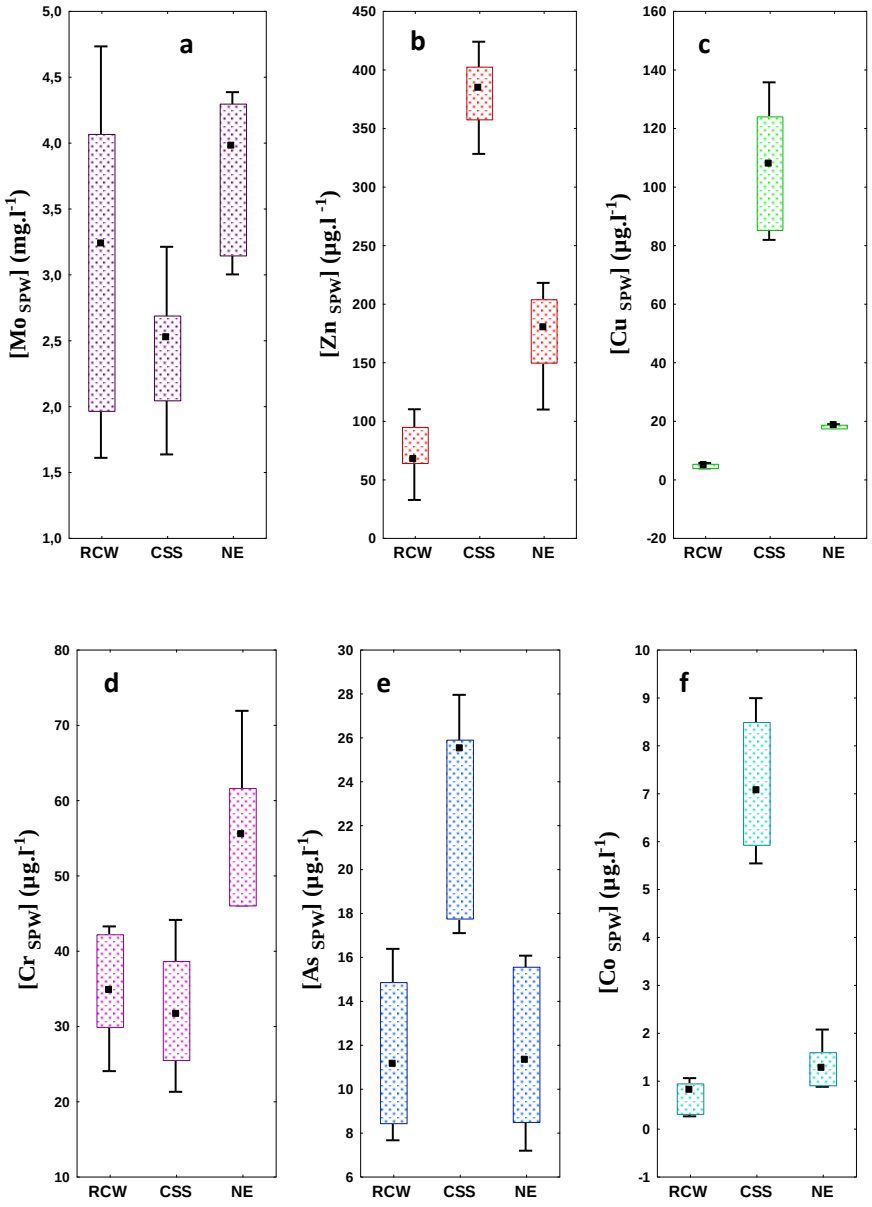

Figure 2. Concentrations of Mo, Zn, Cu, Cr, As and Co in the SPW. Values are ●Median , 25%-75% ± Min-Max (n=9).

3.3. Soil exposure intensity of TE

Soil amendment had not a significant effect on the soil exposure intensity of Zn in the treated soil compared with the soil (NE) (Tab. 4) for both organic amendments The R values calculated from the [Zn $_{DGT}$] devised on [Zn $_{spw}$] measured in the untreated contaminated soil (NE) and the soil amended with CSS was lower than that measured in the soil amended with RCW (Table 4). The increase in the (Zn $_{R}$) values in the three types of soils was as follows: CSS< NE<RCW , where CSS has the capacity to reduce the (Zn $_{R}$) values by about 60%, while the soil amended in the RCW increased it by 51% compared with the NE soil. The higher value in the soil amended with RCW may also indicate highest values of available Zn during the first hours of deployment due to higher initial mobilization flux from the solid phase (Zhang et al., 2001).

The untreated soil (NE) provided the highest concentration of soluble Cr (56.02±9.86 µg.l^{-1}) and also provided the highest concentration of available Cr, this contribution was about 2% of the SPW concentration of Cr. RCW and CSS added to the soil decreased the available concentration of [Cr $_{DGT}$] in the contaminated soils compared with the untreated soil (NE). RCW decreased significantly (p= 0.00001) the available Cr concentration by nearly 73% compared with the NE soil e.g. RCW reduced the available Cr concentration from 0.67±0.10 µg.l^{-1} in the soil without amendment (NE) to 0.18±0.03 µg.l^{-1} in the soil treated with RCW. In contrast in the soil amended with CSS the available concentration of Cr was slightly reduced by 9% compared with the control soil (Tab. 4). The available concentrations of Cr in the soil amended with CSS and in the soil without amendment NE were to about 1.9 % and 0.5% of the SPW concentration of Cr in these soils. Table 4 presents the [Cr $_{DGT}$]: [Cr $_{spw}$] ratio R (Cr $_{R}$) values for all contaminated and uncontaminated soils. The soil amended with RCW presented (Cr $_{R}$) values lower than that presented in the NE soil by 56%, whereas the soil amended with CSS presented the highest (Cr $_{R}$) values compared with the CRW and NE soil. This increase was about 3.8 and 1.7 fold more than that presented in the CRW and NE respectively.

A strong negative correlation was observed between [Cr $_{spw}$] and total and dissolved organic matter in the soil and solution r= -0.82, r= -0.87 respectively, and between [Cr $_{DGT}$] and the electrical conductivity (EC) in the soil r= -0.89. [Cr $_{DGT}$] ,correlation have also showed with total Cr in the soil r=0.68.

We did not detect any available concentration of Cu and Co in the used DGT, thus the ratio calculated from the mobile and available fraction was equal to zero (Table 4).

Table 6: intensity of Zn, Cr, Cu and Co exposure determined by DGT and the Ratios of Zn, Cr, Cu and Co (mean values+ standard deviations) (n=9)

Soils	[Zn $_{DGT}$]	[Cr $_{DGT}$]	[Cu $_{DGT}$]	[Co $_{DGT}$]	(Zn $_{R}$)	(Cr $_{R}$)	(Cu $_{R}$)	(Co $_{R}$)
RCW	27.88±1.77 a	0.18±0.03 a	0	0	0.39±0.04 a	0.005±0.001 a	0	0
CSS	27.31±1.46 a	0.60±0.00 b	0	0	0.07±0.00 a	0.018±0.006 a	0	0
NE	26.07±1.97 a	0.67±0.10 b	0	0	0.162±0.04 b	0.011±0.000 a	0	0
ANOVA	N.S	***	-	-	***	N.S	-	-

3.4. Biomass of primary BL and TE phytoavailability

Table 5 shows the (DW) and a foliar TE concentration of the BL grown on treated and untreated contaminated soils. The beans leaves dry weight (BLDW) in the soil amended with CSS was equal to the one provided by the untreated contaminated soil. In contrast the soil amended with RCW increased the BLDW by 14% and 15% compared to the CSS and NE soils respectively (p=0.013). TE phytoavailability was assessed by measuring TE concentrations in the primary BL. Results presented in Table 5 show that RCW and CSS added to the contaminated soil decreased significantly the concentration of Mo, Cr, As and

Co measured in the BL compared with the soil without amendment (NE) ($p<0.0001$). This decrease in the soil treated with RCW and CSS was about 2.4 and 1.6 fold for Mo, 6 and 3.8 fold for Cr, 2 and 1.8 fold for As and 1.5 and 1.2 fold for Co respectively (e.g. decreased Cr concentration from 14, 46±1.06 ($mg.kg^{-1}$) in the NE soil to 2, 35±0.11 ($mg.kg^{-1}$) in the RCW soil and to 3, 82±0.52 ($mg.kg^{-1}$) in the CSS soil. In contrast the Cu concentration in the BL increased after the addition of RCW and CSS to the soil, but this effect was not significant compared with the untreated soil as this increase was about 10% and 6% respectively (e.g. increased from 14,56±0.21 ($mg.kg^{-1}$) in the NE soil to 16,17±1.28 ($mg.kg^{-1}$) in the soil amended with CSS). Additionnaly the effect of RCW and CSS on Zn concentration in the BL was different of the others TE where we noticed that RCW reduced slightly the Zn concentration in the BL by 7% whereas the addition of CSS had an inverse effect as it increased slightly the Zn concentration by 6.5% compared the untreated soil. A good correlation was found between the foliar Mo, As and Cr concentration and the soil pH measured in these soils ($r \geq 0.7$ and $p<0.05$).

Table 7: DW yields and TE concentrations of bean primary leaves. Values are mean ± standard deviation (n=9)

Sample label	dry weight of beans leaves (g)	Concentration of TE in the leaves beans ($mg.kg^{-1}$)					
		Mo±SD	Zn±SD	Cu±SD	Cr±SD	As±SD	Co±SD
RCW	0,13±0.01 a	14,59±1.02 a	40,85±4.92 a	16,17±1.28 a	2,35±0.11 a	0,26±0.04 a	0,16±0.04 a
CSS	0,11±0.00 b	21,23±0.81 ab	47,03±2.97 a	15,44±0.80 a	3,82±0.52 ab	0,29±0.01 a	0,21±0.1 a
NE	0,11±0.00 b	34,26±3.61 b	43,98±2.34 a	14,56±0.21 a	14,46±1.06 b	0,52±0.03 b	0,24±0.5 a
ANOVA	*	***	N.S	N.S	***	***	NS

* Significant for $p<0.05$, ** high significant for $0.01>p>0.001$, *** highly significant for $p<0.0001$, NS (non-significant).

Figure 3 shows the total TE amount in the primary BL ($\mu g.plant^{-1}$) so-called the mineral masses of Mo, Zn, Cu, Cr, As and Co in the BL calculated based on the foliar element concentration ($\mu g.kg^{-1}$ DW) and leaf biomass production (μg $DW.pot^{-1}$). A higher significant decrease ($p<0.0001$) was found in the mineral masses of the Mo, Cr and As metals in the treated soils (RCW and CSS) compared with the untreated (NE) soil. This decrease in the Mo, Cr and As mineral masses in plants after the addition of RCW and CSS was as follows: 2.0 and 1.6 fold for the Mo , 5.2 and 3.8 fold for the Cr and 1.7, and 1.8 fold for the As and respectivelly. Organic amendments slightly decreased the Co mineral mass by 1.3 and 1.2 fold after the addition of RCW and CSS respectively. However the amendments effects was not signifiant compared for the others TE. In contrast the effect of soil amendement on the Cu and Zn mineral mass was different: the addition of RCW and CSS increased the mineral masses of Cu and Zn by 22% and 4% for the Cu and 8% and 6% for the Zn respectivelly compared with the untreated soil (NE).

If we compared only the effects of organic amendements on the mineral masses of TE, we can see that the soil amended with RCW was more able than CSS to reduce the mineral mass of Mo, Cr and Co in the BL tissues. The RCW decreased the mineral mass of these TE in the BLDW by 19% for the Mo, 27 % for the Cr and 10 % for the Co compared with that calculated in the soil amended with CSS. In contrast RCW was more efficient than CSS to increase the mineral masses of Zn, Cu and As by 2%, 18 and 5% respectively. A strong positive correlation was in fact found between the mineral masses of Mo, Cr, and As except Cu and the soil pH ($r>0.7$, $p<0.05$), whereas this correlation was strongly negative between the same TE mineral masses and the EC measured of the SPW $r > 0.8$, $p=<0.001$ and $r=0.80$, $p<0.05$ respectively.

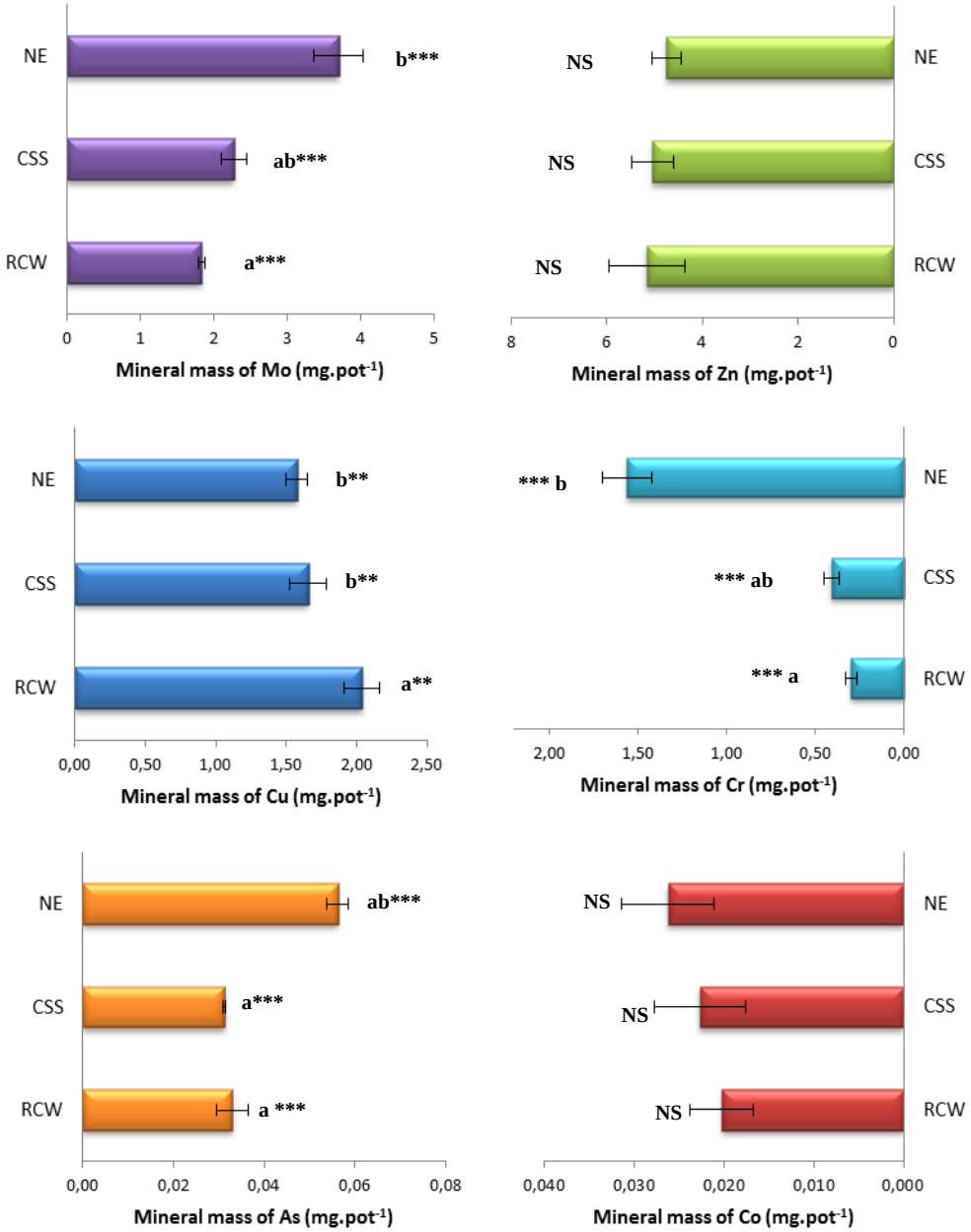

Figure 3: Mineral masses of Zn, Cu, Cr, As and Co in the bean primary leave. Values are mean ± standard deviation (n=9). Different letters on bar graphs indicate a significant difference ($p<0.05$)

4. Discussion

4.1. Mobility of TE

All our results showed that RCW added to our contaminated soil decreased the mobile fraction of TE (Mo, Zn, Cu, Cr and Co) in the SPWmore than CSS compared with the untreated soil (Table 3). Munksgaard and Lottermoser (2010) tested the effect of two amendments on metal mobility in metal-contaminated soils from the Broken Hill mining center, Australia. The first amendment was the Phosphate fertilizer (bovine bone meal, superphosphate, triple superphosphate, potassium orthophosphate) and the second was a fresh organic amendment (pine bark (Pinus radiata)), the two amendments were applied to two soils contaminated with mining waste. The results showed that the addition of fertilizers and/or pine bark to both soil types increased water extractable metals and metalloids concentrations (As, Cd, Cu, Fe, Mn, Pb, Sb, Zn) compared with nonamended soils.

Composted organic wastes such as composted sewage sludge (CSS) amendment have

been shown to have multiple benefits of improving vegetation establishment, reducing compaction, (Bernal et al., 2006), protecting against erosion (Craul, 1999; Whalen et al., 2003) and binding metals (Song and Greenway, 2004); the rapid mobilization and vertical transport of trace metals and As was also a consequence of adding this material to the soil (Beesley and Dickinson, 2010; Beesley et al., 2010).

Clemente et al. (2006) reported that fresh organic wastes, such as municipal waste compost (MWC), are rich in soluble organic compounds which can increase the mobility of Zn by 48% and the Cu by 74% compared with the control soil shortly after the addition of these materials to soil, this increase resulting from the formation of soluble organo-metallic complexes.

These results can confirm our results (Table 3) and explain the increase in the mobile Zn (54%), Cu (83%), Co (82%) and As (49%) concentrations in the SPW after the addition of CSS to the contaminated soil compared with the untreated one. Additionally we noticed that the mobility of TE was strongly related with the dissolved and total organic carbon concentrations measured in the soil and soil solution. Moreover, the composition of organic amendments can change with time due to decomposition of organic matter by soil microorganisms, with soluble organo-metal complexes being released into soil solution (Hooda and Alloway, 1994; Kaschl et al., 2002). Xia et al., 2005 tested also the effect of varying amendment rates of compost on the mobility and the leaching potential of TE in the soil. They found that increasing compost rates significantly increased the extractable fraction of Cu and Zn by 8 fold and 7 fold respectively at 1 $L.L^{-1}$ of compost compared with the soil without compost (0 $L. L^{-1}$).

In conctrast CSS added to our contaminated soil increased the mobile fraction of Cu and Zn by 6 and 2.2 fold respectively compared with the soil without amendment. Beesley et al., 2010 found that applying amendments to multi-element contaminated soils can have contradictory effects on the mobility, bioavailability and toxicity of specific elements, depending on the amendment. TE were measured after 60 days in a contaminated soil amended with biochar and green waste compost, after that phytotoxicity was assessed by a simple bio-indicator test. They found that Cu and As concentrations in the SPW increased more than 30 fold after adding both amendments, associated with significant increases in dissolved organic carbon and pH, whereas Zn and Cd significantly decreased. Gigliotti et al., 1996 evaluated the accumulation of TE in the top 20 cm of calcareous soil amended with urban waste compost. They reported compared with untreated soils that, amended soils showed a significant increase only in Cu, Zn, Pb and, in the last 2 years, Cr concentrations. They concluded that the long-term application of large amounts of urban waste compost to $CaCO_3$-containing soils does not necessarily cause medium-term problems to plant, animal or human health. Two types of composts were tested as mulching materials in a vineyard by Pinamonti, (1998): one was sewage sludge and bark (SB) compost with a low trace elements concentration, the other was a municipal solid waste (MSW) compost with a higher concentration of metals. Both compost mulches ameliorated soil properties; they increased organic matter content and improved the porosity and water retention capacity of the soil. The SB compost only caused a significant increase in total and DTPA-extractable Zn in the soil; with this compost the soil concentrations of the other trace elements examined (Cu, Ni, Pb, Cd and Cr) did not show significant changes. In contrast, the MSW compost, used over a six-year period, increased concentrations of total Zn, Ni, Pb, Cd and Cr and of DTPA extractable Zn, Ni, Pb and Cd in the soil when compared to the control. Cao et al 2003 investigated the effects of phosphate rock, municipal solid wastes (MSW) and Biosolids (BS) on the leaching of As from soils and As uptake by Chinese brake fern in a chromated–copper–arsenate (CCA) contaminated soil or in As spiked contaminated (ASC) soil. They found that As uptake in the compost-treated CCA soil was related to the increase of soil water-soluble As and As(V) transformation into As(III). Reduced As uptake in the ASC soil may be attributed to As adsorption to the compost. Compost and phosphate amendments increased As leaching from the CCA soil by 34% and 26% compared with the control soil, but had decreased leaching with the presence of As accumulator (Chinese brake fern (Pteris

vittata L). These results confirm the effect of CSS which increased the mobile concentration of As by 49% compared with the untreated soil. Shuman (1999) tested the effect of five organic wastes or humic acid on two types of soils (fine and coarse textured), he found that a wide range of organic materials tested (except poultry litter) increased Zn adsorption, mainly for sandy soils. In our experiment, we found an inverse effect of CSS on the Zn mobility, where the addition of CSS to the soil increased the mobile fraction of Zn by 54% compared with the untreated soil. Narwal and Singh (1998) stated that the effects of organic amendments on Zn availability were related to soil pH. However this was not shown in the present experiment, where we did not notice any difference on available concentration of Zn between the control soils which contain ph higher than the others amended soils. The ecological effects of metals and metalloid in soils are closely related to the concentration and speciation of the elements in the soil and soil pore water (Bruemmer et al., 1986). Generally the plants exposure to metals is related to the concentrations of both mobile and available trace metals in SPW (Sauvé et al. 1997; Sauvé 2003; Tandy et al. 2006; Forsberg et al. 2009). Organic matter additions to soil have long been considered important in maintaining the quality of both natural and managed soils. When RCW decomposes on a soil, stable and enduring humus is created. The lignin's in the RCW are slowly broken down by naturally occurring fungi, and these fungi also play a vital role in the soil food web, serving as nourishment for microorganisms (bacteria, fungi, and soil fauna), which well rapidly invades the RCW according to specific stages of succession (Käärik, [1974]). The second organic matter which we used is composted sewage sludge (CSS), which provides soil organic matter more mature than the previous.

The majority of trials showed highest soil organic matter (SOM) effects on soil properties especially with composted material. Only few experiments could not differentiate these effects between the C sources (straw, manure, compost). However, well matured compost leads to a higher SOM increase than fresh compost. The stable C fraction responsible for OM reproduction is highest in mature compost (50% of total compost C). This increase in soil organic matter is due to the increased amount of organic matter present, and also due to the increased biological activity within the soil due to the fresh pool of organic substrate SOM has probably the greatest capacity and strength of bonding with most trace metals of any soil component (the possible exceptions are some non-crystalline minerals with very high surface areas). As a consequence there are often statistically significant correlations between solubility of trace metals such as Cu, Hg and Cd, and Soil Organic Matter Content. Generally the metals that bond most strongly to SOM tend also to be the most rapidly adsorbed. When these metals such as Pb^{3+}, Cd^{2+}, Cu^{2+} and Fe^{3+} complexes with soil organic matter has low lability. In contrast when few trace element such as Cu^{2+}, Ni^{2+} and Co^{2+} form with dissolved humic and fulvic acid, acid-metal complexes, it appear to be largely labile. This lability is controlled by the soil pH and the metal/organic ratio, decreasing as pH is raised and as the metal/organic ratio is decreased (Petruzzelli & Pezzarossa, 2003; Leita et al., 2003).

4.2. Phytoavailability of TE

Organic amendments added to the soil (RCW and CSS) reduced Mo, Cr, As and Co concentrations in the beans grown in treated soil compared to the untreated soil which exceeded for many TE the limits values reported by (Kabata-Pendias and Pendias, 2000) for foliar Cu, Cr, As and Zn concentrations, these limits values are 20, 5, 5 and 100 mg. kg^{-1} DW, and that reported by MacNicol and Beckett, (1985) are 15-30 Cu µg Cu g^{-1} DW, < 1 µg Cr g^{-1} DW Cr, 60-250 µg Zn g^{-1} DW and 1-4 µg As g^{-1} DW.

Addition of fresh organic material (RCW) to the soil decreased the mobility of Zn, Cr, Cu and Co measured in the soil solutions (Fig. 2) and the TE concentrations such as (Mo, Cr, Zn, As and Co) measured in the BL, except for Cu (Table 4).

Angelova et al (2010) investigated the effect of several organic amendments (peat, compost and vermin compost) on the quantity of mobile forms of Pb, Zn, Cd and Cu and uptake of

these elements by potato (Solanum tuberosum L.) plants. They concluded that the application of soil amendments favors plant growth and its development which confirmed by our results in the case of CSS whereas RCW didn't show any effect; these effects were best expressed with the application of 10% compost (5% in our experience). They also found that organic amendment application led to an effective immobilization of Pb, Cu, Zn and Cd phytoaccessible forms in soil and to decreased the trace metal content in the potato peel and tubers. This agrees with that we found after RCW addition except for the Cu, and expect Cu and Zn after CSS addition. Gigliotti et al., 1996 investigated the effects of soil amended with urban waste compost on the uptake and distribution of TE (Cd, Cu, Ni, Pb, Zn and Cr) in corn plants grown in this soil. They reported that the corn plants grown on the amended soil showed a general increase in metal uptake, which was about three times greater for Pb and two times greater for the other metals than in plants grown on untreated soil. They also found that Cu and Zn in all plant parts and although the quantity absorbed by the plants from the treated plots was higher, their distribution was uniform in all plants. These results agree with the effect of CSS added to our soil in Cu and Zn concentration in the leaves beans which increased by about 6 and 6.5 % compared with the control soil. Businelli et al., 1996 investigated the effect of two types of compost (MWC) on the mobility and the availability of (Cu, Pb, Zn, Cd, Cr and Ni) in argillaceous loam calcareous soil for 6 years. They found that amendments increased significantly the mobile TE concentrations in the treated soil compared with the untreated one.Cu, Pb, and Zn measured in the plant grown on the amended soil were generally higher than those measured in the control soil. This was not however the case for Cd, Cr and Ni measured in the treated soil. These results confirm exactly our results on the effects of CSS, where this amendment increased Cu and Zn concentration in the BL, whereas it decreased the Cr concentration by 3.8 fold compared with the untreated soil. Bes and mench. (2008) added five organic matters (three phosphate compounds, zerovalent iron grit (ZVIG, 2% by soil weight), two alkaline compounds, and two commercial formulations were incorporated, singly and some combined with ZVIG, into a highly Cu-contaminated topsoil (2600 mg Cu kg^{-1}) from a wood treatment facility. They found that the most of amendment enhanced the growth of beans in treated soils compared to the untreated, CSS and Compost poultry manure (CPM) added to the contaminated soil increased the quantity of the biomasses by about 1.5 fold and 1.4 fold respectively compared with the untreated soil. In our experiment CSS did not have any effects on the biomass of the BL compared with the untreated soil, whereas RWC slightly increased the BL biomass by 1,2 fold compared with the untreated soil. Cu and Zn concentration measured in the BL decreased by 1.5 and 2 fold respectively while we found that addition of CSS to our contaminated soil increased the concentration of Cu and Zn by 6 and 6,5 fold compared with the untreated soil. However the soil type and Cu concentration range was very different from our study. Bes and mench. (2008) reported that the Cu mineral mass [Cu_{MM}] increased in the soil amended with compost of sewage sludge (CSS) and a mixture of Compost poultry manure and zerovalent iron grit (CPMX ZVIG), whereas our results showed that there was any significant effect of amendment on the mineral masses of Cu in the beans leaves.Castaldi and Melis, 2004 reported that organic amendments (compost) contain a high proportion of humified organic matter, which could decrease the TE bioavailability in soil, decreased finally its toxicity for the vegetation. Addition of fresh organic material is usually associated with the arrival of a large number of microorganisms and macroorganisms to the soil. Interactions between microorganisms and metals are multiple: accumulation, intracellular, combination metal/ cell wall, interaction extracellular polymeric / metal, etc.... These reactions can lead to in theory to immobilization or remobilization the trace elements in the soil under certain environmental conditions (Ford and Mitchell, 1993). The bacteria in soil possess surfaces that interact strongly with metal ions in soil solution could adsorb a greater amount of heavy metals than inorganic soil components such as montmorillonite, kaolinite or vermiculite (Ledin et al., 1996), since bacterial cells (approximately 1.0e1.5 mm3) have an extremely high ratio of surface area to volume, which endows bacteria with a strong capacity at adsorbing and

immobilizing toxic ions from soil solution (Beveridge and Schultze-Lam, 1995).

5. Conclusion

First the mobility of TE was assessed by investigating the total dissolved concentrations of TE in the SPW of the amended and control soils. Total dissolved TE concentrations were determined in soil pore water (SPW) sampled by Rhizon samplers. The average results of each 3 plots showed that RCW did significantly reduce the Mo, Zn, Cu, Cr and Co dissolved concentrations in the SPW whereas it did not have any effect on the As concentration. In contrast the application of CSS significantly increased the dissolved concentrations of Zn, Cu, Co and As in the SPW while it decreased for Mo and Cr. Thus RCW decreased the mobility of all studied TE in the SPW, whereas CSS reduced the mobility of Mo, Cr and Co, while it increased the mobility of Zn, Cu and As. Second the soil exposure intensity was assessed by DGT probes. The Zn soil exposure intensity assessed by DGT were not significantly changed by the addition of RCW and CSS, while the Cr soil exposure intensity was significantly decreased after RCW addition compared with the soil treated with CSS and the NE soil. Cu and Co could not be detected by DGT in our three soils suggesting these TE were non labile. Third the TE phytoavailability was characterized by growing dwarf beans on potted soils and analyzing their foliar TE concentrations. The effect of organic amendments on the TE concentration in the beans leaves was very important: we found that the soil amended with RCW provided a bean leave dry weight higher than the others soils and RCW was able to reduce Mo, Zn, Cr, As and Co concentration in the bean leaves compared with the NE soil. RCW was also more efficient than CSS to reduce the mineral mass of Mo, Cr and Co in the beans leaves tissues. In contrast CSS was more able than RCW to increase the mineral masses of Zn and Cu in the bean leaves . Consequently, RCW was more effective than CSS to reduce TE mobility and phytoavailability in the contaminated techno-soils.

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Chapitre 2.4.

Effet des amendements organiques sur la mobilité des éléments traces dans un techno-sol contaminé phytoremédié: rôle des substances humiques

2.4.1. Résumé de la publication

L'article précédent a présenté le travail réalisé sur un technosol de Châteauneuf avec pour objectif d'évaluer l'efficacité de la phytostabilisation assistée par des amendements à remédier ce site contaminé en forte concentration d'ET. Les résultats de l'article précédent ont montré que les deux amendent ont des effets positifs, notamment dans le cas du RCW.

Ce travail a été fait sur le même site (techno-sol) à fin de caractériser le rôle des substances humiques (acides fulviques (FA) et acides humiques (HA)). Les extraits FA et HA ont été réalisées sur les mêmes sols après phytostabilisation assistée par des amendements organiques tels que le bois raméal fragmenté (BRF) et les boues d'épuration compostées (CSS). Il s'agit d'évaluer le rôle de l'humification liée à la phytostabilisation des ET en mettant l'accent sur l'As, Cu, Mo, Pb et Zn.

Les travaux ont porté sur neuf parcelles traitées par phytostabilisation avec des graminées et avec différents types d'amendements. Les neuf parcelles sont: (A) 3 parcelles de sols non amendés (NE), (B) 3 parcelles de sol amendés avec des matières organiques fraiches comme le bois raméal fragmenté (RCW) et (C) 3 parcelles de sols traités avec boues d'épuration compostées (CSS) ayant une grande maturité. Sur les trois premières parcelles de chaque groupe A, B et C, un mélange de plantes classiques a été semé; sur les trois parcelles suivantes, des plantes métallicoles ont été cultivées et en fin des plantes non-cultivées se sont développées sur les trois dernières parcelles.

L'analyse Rock-Eval a été menée pour estimer les indices HI, OI et la teneur en carbone organique total (TOC). Les substances humiques (HSS) ont été extraite avec 500 ml de NaOH 0,5 N (Schnitzer et Schuppli, 1989). Les acides humiques et fulviques utilisées ont été extraits par la procédure donnée par Holtzclaw et al., (1976). Le TOC de chaque solution a été déterminée par combustion catalytique à chaud (Teckmar - Dohrman Phoenix 8000). Le contenu total en ET dans les FA et dans le mélange de FA + HA a été déterminé par l'ICP-MS. Les spectres d'absorption ultraviolet-visible (UV-VIS) de l'acide fulvique ont été utilisés pour calculer les ratios E4/E6 et E2/E3. E2/E3 est considéré comme un indicateur de la taille moléculaire et de l'hydrophillicité des AF ; tandis que le rapport E4/E6 est inversement relié à leur degrès d'aromatisation et leur poids moléculaire.

Les résultats ont montré que l'ajout de CSS au sol a augmenté le carbone organique total et la teneur en azote plus qu'avec l'ajout de RCW par rapport au sol sans amendement (NE). Le rapport C/N dans le sol CSS était plus élevé que dans le sol amendé avec RCW et le sol sans amendement NE, ce

qui suggère une plus grande décomposition de la matière organique (SOM) dans le sol CSS par rapport aux autres sols.

Le rock eval a montré aussi l'origine de la matière organique présentent dans nos sols. Il a montré l'origine terrestre de la matière organique du sol sans amendement. Il montre également que les valeurs obtenues dans les sols amendés sont très dépendantes de celles de l'amendement lui-même. Les copeaux de bois Raméaux (RCW) augmentent l'indice IO en raison de la présence de la cellulose de bois. Le sol amendé avec les boues d'épuration compostées (CSS) a un IH plus élevé parce qu'il est plus riche en groupes aliphatiques que le bois. L'ajout de CSS au techno-sol a augmenté également de façon significative ($p < 0,001$) le pourcentage de carbone organique associé aux fractions HA par apport aux sols RCW et NE. Le sol amendé avec CSS possède le rapport E4/E6 le plus élevée et le ratio E2/E3 le plus faible. Ces variations sont directement liées à la maturation de l'amendement. Zn et As sont plus abondants dans la fraction de FA que dans la fraction de HA. En revanche Pb, Cu et Mo sont plus associée à HA que à la fraction FA dans les sols traités et non traités, ce qui peut expliquer la différence dans la mobilité et la disponibilité de Zn et As par rapport les autre métaux comme Pb, Cu et Mo. L'effet des amendements sur la mobilité des ET a varié d'un ET à l'autre.

2.4.2. Article 4 (Soumis à Environmental pollution)

Effect of organic amendments on the mobility of trace elements in phytoremediated technosoils: role of the humic substances

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Abstract: The efficiency of aided phytostabilization using organic amendments such as ramial chipped wood (RCW) and composted sewage sludge (CSS) was studied on contaminated techno-soils, on nine experimental plots. The objective was to characterize the role of fulvic (FA) and humic acids (HA) on mobilization of trace elements with emphasis on As, Cu, Mo, Pb and Zn.

Results showed that addition of CSS to the soil increased the total organic carbon and nitrogen content more than that amended with RCW and as a results, the C/N ratio in the CSS soil was higher than in the RCW and non amended (NE) soil, which reflect the high decomposition of organic matter (SOM) in the CSS soil compared with the others soils. The RCW and CSS amendments increased the *hydrogen index (*HI) values and the oxygen index (OI) values compared with the NE soil, especially for the soil treated with CSS which contained more aliphatic than aromatics compounds. The addition of CSS to the techno-soil increased significantly ($p<0.001$) the percentage of C_{org} associated with the HA fractions compared with the RCW and NE soils. The soil amended with CSS showed the highest E_4/E_6 ratio and the lowest E_2/E_3 ratio of FA extracted from the soil amended with CSS. Zn and As are more abundant in the FA fraction than in the HA fraction. In contrast Pb, Cu and Mo were more associated to HA compared with FA in the treated and untreated soils, which can explained the difference in the mobility and the availability of Zn and As compared with Pb, Cu and Mo.

Key words: Contaminated techno-soil, Trace element, Humic acid, Fulvic acid, Mobility.

1. Introduction

Techno-soils are considered one of the soils the most exposed to pollution from the anthropic soils (Bulgariu et al., 2007; Thornton, 1991; Florea and Munteanu. 2003; IUSS, 2006). The pollution of anthropic soils with high concentration of trace elements is more problematic than for other types of soils, as the properties of these techno-soils changed with the time, making handling more difficult (Kelly et al., 1996). Moreover they present a real risk for human health and especially for children's health (De Burbure et al., 2006).

The contamination of a techno-soil with trace elements requires special soil-management techniques to limit the trace element solubility and thus plant availability. Among remediation techniques, the phytoremediation techniques, which have attracted attention as a low-cost and ecologically sustainable alternative to physicochemical methods, are applicable to large areas and accepted by local populations (Mench et al., 2010). Aided phytostabilization is one of phytoremediation techniques, which is recognized as a potentially cost-effective and ecologically sound approach to containment of trace element-polluted soils and mine tailings. Its principal objective is to reduce the mobility, ecotoxicity, and

dispersion of metals/metalloids through the environment (Mendez and Maier, 2008; Ginocchio et al., 2007; Mench et al., 2010). Aided phytostabilisation technique requires the incorporation of both plant species and organic or inorganic amendments adequate with the contamination type for the immobilizing of trace elements such as metals/metalloids and reduce their availability in these soils (Mendez and Maier, 2008; Berti and Cunningham, 2000) and to restore the physical, chemical and biological properties of contaminated soils (Mench et al., 2000; Bolan et al., 2003; Pérez de Mora et al., 2005; Raicevic et al., 2005; Kumpiene et al., 2006; 2008). Several mineral and organic amendments such as lime, coal fly ashes, phosphates, red muds, compost, biosolids, iron grit and Fe/Mn/Al oxides can improve phytostabilization and the production of plant-based feedstock through the decrease of the solubility, leaching and bioavailability of trace elements (Lombi et al., 2002; Bolan et al., 2003; Brown et al., 2004; Geebelen et al., 2003; Basta et al., 2004; Kumpiene et al., 2008; Mench et al., 2010) through various complex processes, e.g. adsorption onto mineral surfaces, formation of stable compounds with organic ligands, surface precipitation and ion exchange (Kumpiene et al., 2008; Ahmad et al., 2011).

Addition of organic matter amendments such compost, manure or various organic wastes with the plants is a common practice for the remediation of contaminated soils. The effect of organic matter amendments on trace element bioavailability depends on the nature of the organic matter, their microbial degradability, salt content and effects on soil pH and redox potential as well as on the particular soil type and metals concerned (Walker et al., 2003; 2004). Indeed, organic amendments may contain a high proportion of humified organic matter, which has a large capacity to interact with metal ions and mobilize or immobilize trace elements through the formation of more or less stable complexes. The formation of these insoluble metal compounds reduces their mobility through the soil profile and the pool available for biota (Geebelen et al., 2003). Characteristics of humus fractions have proven useful in the interpretation of organic matter dynamics in soil. In fact a lot of literature exists on the characterization of humic substances (HSs) (Abbt-Braun and Frimmel, 1999; Gondar et al., 2005; Shirshova et al., 2006) and on binding of metals to humic substances mainly concerning laboratory experiments with humic or fulvic fractions purified from water or soil (Vaca-Paulín et al., 2006; Gondar et al., 2006; Doig and Liber, 2007; Evangelou et al., 2007). The ability of humic substances to react with trace elements has long been recognized (Wu et al., 2002; Clemente and Bernal, 2006; Evangelou et al., 2007). Humic substances have indeed the capacity to control the behavior of trace elements in the environment. However the reaction between trace elements and humic substances are highly complex and depend on the trace element itself, humic material and pH. There are two possible outcomes of humic-metal binding: (i) metal ions may form soluble organic complexes with the potential to contaminate ground water and to retain the metal in soil solution; and (ii) insoluble complexes can form and result in a reduction in bioavailability and consequently, ecotoxicity of the metals (Logan et al., 1997).

In the present work we assess the role of humic substances (Humic acid and Fulvic acid) extracts from two different organic amendments soils [composted sewage sludge (CSS) and fresh ramial chipped wood (RCW)] and from an untreated soil on the mobility of several trace elements in a metallurgical techno-soils treated by aided phytostabilisation.

2. Materials and methods

2.1. Site description and experimental design

The studied site is a metallurgical landfill (Industeel-Loire; 45°32’ N; 4°38’E) located near Lyon. France. It is located behind a steel and iron factory which is still in activity. The site has been used from about 1850 to 2001 to dispose of foundry wastes, slags, fire-bricks, and other by-products from the industrial process, such more or less hydrated lime. It extends over nearly 15 ha and 8 to 10 m thick. The soil (“foundry technosol” type) is highly contaminated with iron (Fe) and different metals used in various alloys: mainly molybdenum

(Mo), arsenic (As), copper (Cu), lead (Pb) and zinc (Zn). This site is part of a French network of contaminated sites ("SAFIR" network. http://www.safir-network.com) and is partly dedicated to researches about contaminant transfer, biological impact of pollution and remediation technologies.

In this context, the efficiency of aided phytostabilization using organic amendments was studied on nine 50 m^2 (5 m X 10 m) experimental plots. Plots were prepared in December 2009 by a shallow ploughing (10 to 15 cm depth) and they were enriched using the following materials: (1) ramial chipped wood (RCW) applied at approx. 500 m^3/ha (3 plots), (2) composted sewage sludge (CSS) applied at approx. 120t DM/ha (3 plots) and (3) no organic enrichment (NE) (3 plots). An extensive pedo-geochemical characterization of the different plots was performed in March 2010, i.e. four months after plots preparation.

The soils were air-dried and sieved to < 2mm. The main pedagogical characteristics of the soils studied (taken at 0-20 cm depth), and their chemical composition are given in table 1. The soil pH was measured in 1:2.5 soil: water suspension using a glass electrode pH meter (NF ISO 10390 procedure (AFNOR. 1994)). Total organic carbon (TOC) was determined in the soil samples by Rock-Eval. This instrument uses a ramped temperature pyrolysis technique where a small amount of sample (70 -100 mg) is heated in an inert atmosphere (helium or nitrogen) and also combusted with air to obtain several key geochemical parameters such as the total organic carbon (TOC). Total N contents were determined using a Fisons Instruments (Crawley. UK) elemental analyzer model EA 1108. The cation exchange capacity (CEC) was determined by the Metson method at pH=7. Particle size distribution of <2 mm fraction was determined by sieving and using the pipette method. Major and metal extraction was performed using hydrofluoric (HF) and perchloric ($HClO_4$) acids, according to the NF X 31-147 procedure. Contents of major and metallic elements in various extracts were measured by inductively coupled plasma optical emission spectrometry (ICP-OES) using a Horiba Jobin-Yvon apparatus.

2.2. Characterization of soil organic matter by the Rock-Eval pyrolysis

The Rock-Eval analysis (Vinci Technologies, Rock-Eval 6 Turbo, France) was developed for estimating the petroleum potential of sedimentary rocks: IH, OH and Tmax, the total organic carbon content (TOC), the total mineral carbon content (CMin) by heating samples in an open pyrolysis system under argon (Behar et al., 2001, Disnar et al., 2003; Sebag et al., 2006). This technique can be applied to soils (Di Giovanni et al., 1998). An analysis takes place in 2 steps. First, during a pyrolysis under nitrogen a desorption of hydrocarbon is maintained at 200 °C for 5 min. Then a cracking occurs between 200 and 650 °C at 30 °C min^{-1}. Hydrocarbons are monitored by a flame ionization detector (FID), forming the so-called peak S1 (mgHc $(g_{sample})^{-1}$, 'volatilized hydrocarbons'), S2 (mgHc $(g_{sample})^{-1}$, 'pyrolyzed hydrocarbons'). At the same time infrared cells detect CO and CO2 (Rock-Eval gives 2 values: S3CO (mgCO $(g_{sample})^{-1}$) and $S3_{CO2}$ (mgCO2 $(g_{sample})^{-1}$) corresponding to the release of functions linked to organic molecules. At higher temperature it records also the $S3'_{CO}$ which is organic and mineral CO and S3' generally attributed to mineral CO_2. In the present work, organic CO_2 was integrated up to 550 °C for the soil. Second, the residue recovered after pyrolysis undergoes a combustion from 400 °C up to 850 °C, under artificial air (N_2 : O_2, 80 : 20) in a second oven. CO and CO_2 released are monitored on line by means of infrared cells. The Rock-Eval method then gives $S4_{CO}$ and $S4_{CO2}$ (respectively mgCO $(g_{sample})^{-1}$ and $mgCO_2$ $(g_{sample})^{-1}$) of organic origin in this work. Finally, S5 measure the CO_2 provided by the mineral source above 400 °C. The initial weight was measured with a precision over 0.5 mg in order to obtain reliable Rock-Eval parameters (*i.e.*, S2 $\pm$ 0.5 mg $(g_{rock})^{-1}$, TOC and C_{Min} $\pm$ 0.1 wt %). In this work only the TOC and HI vs OI were considered as the main parameters following the original work

of Van Krevelen (D.W. Van Krevelen, Fuel, 29(12); 1950:269-92).

2.3. Extraction and characterization of humic substances (HSs)

One hundred grams of each soil sample was extracted with 500 ml of 0.5N NaOH (Schnitzer et Schuppli, 1989). The humics and fulvic acids used were extracted by following procedure given by Holtzclaw et al., (1976). The mixture was stirred for 17h under N2 gas. Humic acids were precipitated with 6 M HCl (pH 1) and separated by centrifugation (3000 rpm. 15 min). Total organic C of each solution was determined by hot catalytic combustion (Teckmar – Dohrman Phoenix 8000).

Total metal contents in FA and in the mixture of FA+HA were determined by HR-ICP-MS (Element 2. Thermo fischer. Waltham. USA) using a Twinnabar cyclonic spray chamber with a Micromist nebuliser (Glass Expansion. Melbourne. Australia) for As, Cu, Mo, Pb and Zn.

The ultraviolet-visible (UV-vis) absorption spectra of separated FA and HA were recorded by a Jasco V-530 spectrophotometer. The E_4/E_6 ratios were calculated as the ratio of absorbance at 465 and at 665 nm according to Chen et al (1977). The E_2/E_3 ratios were calculated as the ratio of absorbance at 280 and at 365 nm. SUVA index is calculated by dividing the UV absorbance measured at λ= 254 nm by the TOC concentration (Weishaar et al., 2003).

Infrared characterizations in the region of 400 - 4000 cm^{-1} were undertaken in transmission mode for several samples in classical pellets forms. The preparation of the pellets was as follows: the sample was finely ground in an agate mortar and 1 to 1.5 mg of the resulting powder was mixed with KBr, previously dried at 120 °C for 24 h. to make a 150 mg pellet. The mixture was homogenized and pressed in an evaluable die to prepare a 13 mm diameter pellet. The operating conditions were 256 scans, 2 cm^{-1} resolution over the 400 – 4000 cm^{-1} range without ambient H_2O and CO_2 correction.

2.4. Statistical analyses

All statistical analyses of data were performed using StatSoft Statistica (version 6). All analytical determinations were performed in three replicates for each plot. Differences were considered statistically significant at $p < 0.05$.

3. Results and discussion

3.1. Physico-chemical properties

The physico-chemical properties of these samples are presented in Table 1. The studied soils are alkaline (pH between 9.08 to 9.34), sandy and with CEC ranging from 5.63 to 6.77 cmol/kg. As expected, applications of the organic amendments have slightly modified the physico-chemical properties of the soil (Larney and Angers, 2012). A significant decrease in pH and an increase in the CEC are observed. In case of carbonated soils, Séré et al. (2010) show that application of organic amendments as compost can lead to a decrease of pH by soil decarbonitization. The increase of the CEC can be explained by the increase of the total organic carbon (TOC) measured in treated soils (p=0.00037) compared with untreated soil (NE). Indeed, soil amended with RCW and CSS presented 2.2 and 3.5 fold of total organic carbon more than that presented in the NE soil. TOC in the soil treated with RCW was less than that measured in the CSS soil, this could be due to microbial decomposition of carbon and its subsequent release as CO_2 (Basiramakenga and Simard, 1998). Moreover, Soumane et al. (2003) showed that the increase of the concentration of organic matter is related to the decomposition of organic amendments in soil and ramial chipped wood decompose slower than compost sewage sludge. RCW and CSS added to the soil increased significantly the total nitrogen by 1.6 and 7 fold respectively compared with the NE soil (p=0.0047) (Table 1). This results agree well with that found by Vaca-Paulın et al. (2006), who reported that the addition of compost and sewage sludge increased the total nitrogen in the soil by 3 fold compared with the control soil. Our results are also in good agreement with those of Kaschl et al. (2002) who investigated the effect of compost added to a calcareous soil on the

characteristics physicochemical of the soil. They found a positive correlation between the addition of compost and soil organic carbon contents. Crecchio et al. (2001) reported also that amendment with municipal compost increased the total C and N in the soil significantly compared with the others treated and control soils. RCW added to the soil increased the C/N ratio by 1.4 fold, whereas the addition of CSS reduced the C/N ratio by 1.9 fold compared with the NE soil. The decrease in the C/N ratio in the CSS soil reflects the high microbial activity, which leads to high decomposition of organic matter (Hsu and Lo, 2001; Amir et al., 2005) and a degree of humification larger.

Table 8: The main pedological characteristics and chemical composition of the studied soils. For each treatment, data are the mean of 30 measures (10 samples by plot).

		Treatment		
		RCW	**CSS**	**NE**
	Texture	sandy	sandy	sandy
physico-chemical parameters	pH	9.08±0.07	9.07±0.12	9.34±0.18
	C_{org} (g.kg^{-1})	19.7±0.34	31.4±0.34	8.9±0.24
	N (g.kg-1)	0.9±0.03	3.9±0.13	0.6±0.01
	C/N	22.67±4.31	8.45±2.01	15.76± 4.73
	CEC (cmol/kg)	5.63±1.10	6.77±0.15	5.77±0.55
majors elements	**P_2O5 (g.kg^{-1})**	0.03±0.00	0.25±0.04	0.02±0.00
	K_2O (g.kg^{-1})	0.29±0.05	0.59±0.07	0.09±0.07
	CaO (g.kg^{-1})	18.88±6.71	20.70±1.13	24.67±2.17
	MgO (g.kg^{-1})	2.66±0.44	1.29±0.09	2.79±0.44
	Na_2O (g.kg^{-1})	0.08±0.07	0.14±0.02	0.14±0.03
trace elements	**Zn (mg.kg^{-1})**	1719.00±777.78	1348.33±560.02	1326.00±739.13
	Pb (mg.kg^{-1})	688.33±453.8	623.00±246.43	839.00±390.20
	Mo (mg.kg^{-1})	523.66±142.30	652.66±69.28	682.33±124.44
	Cu (mg.kg^{-1})	399.00±84.01	410.33±78.23	523.00±160.36
	As (mg.kg^{-1})	76.66±22.47	73.00±11.78	91.33±15.54

3.2. Organic geochemistry

Rock Eval analyses of the treated (RCW and CSS) and untreated soil (NE) was conducted in order to recognize the origin and the evolution of the MO contained in each soil. Examination of the results obtained leads to the following conclusions: (1) the TOC content in treated soils was significantly higher than that measured in the untreated one (Table. 1); (2) the same behavior was marked for the hydrogen index (HI) where RCW and CSS presented 63% and 75% of HI more than the NE soil. However, addition of organic amendment to the soil increased slightly soil Oxygen Index (OI) by 23% and 13 % in soil amended with RCW and CSS respectively (Table. 2). The HI values presented by the soil amended with CSS were nearly 2 fold higher than that presented by RCW, whereas RCW presented 1.8 fold of OI higher than that present by the soil amended with the CSS (Table. 2). This result is in agreement with the low organic content in the untreated soil (low TOC) and thereafter the expression of the nature of the two organic amendments in the RCW and CSS respective values: their addition in the soil increases the TOC and the IH index (RCW). Thereafter a very strong correlation was observed between TOC and HI measured in the 3 types of soils (r= 0.94). Our results are in agreement with that reported by Walter et al. (1982) who found very good correlation between TOC content and the kerogen hydrogen index.

Table 2: Pyrolyse Rock-Eval. Carbone Organique Total (TOC. %). Indice Hydrogène (HI. mg HC.g^{-1}TOC). Indice Oxygène (OI. mg O_2 g^{-1}TOC)

	TOC (g/100g)	HI	OI	HI/OI
RCW	1.97ab±0.34***	181.41ab±15.08***	188.86a±23.84 NS	0.965
CSS	3.14a±0.34***	264.54a±11.50***	165.52a±7.58 NS	1.6005
NE	0.89b±0.24***	66.01b±13.43***	143.67a±28.99 NS	0.4593

The relation between the hydrogen and oxygen index values has been expressed through the IH and IO diagram (Fig. 1). This figure allows to present the three types of our soils compared to the well-known Van Krevelen diagram. This curve consists of three lines highlighting the origin natural of the organic matter on the soil (Tissot and Welte, 1984; Espitalié et al., 1977; Hassen et al., 2009). Lines I and II correspond to organic materials of microbial or planktonic algal; line III corresponds to the MO continental higher plants.

The most immediate explanation is based on the fact that the position of the points representing of our treated and untreated soils is along lineage III. That shows the terrestrial origin of the organic matter of the untreated soil. It also shows that the values obtained in the amended soils are very dependent on that of the amendment itself. Ramial chipped wood (RCW) has a higher IO index because of the presence of cellulose of wood. As the original kerogen of the non-treated soil, is not abundant and also because its IO index is low, RCW soil has the highest IO, close to that of wood. Composted sewage sludge (CSS) has a higher IH because it is richer in aliphatic groups than wood. This is in relation with its origin. Thereafter the soil amended with CSS has a higher IH value than the others.

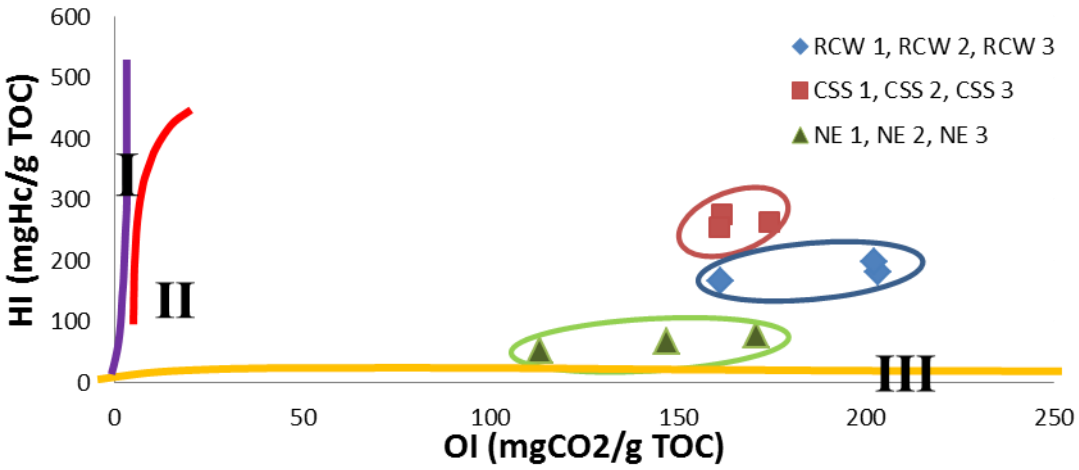

Figure 1: Scatter diagram of hydrogen and oxygen indices for organic matter in soil amended with RCW and CSS and untreated soil NE.

1.3. Characterization of the humic fraction

Humic substances (fulvic and humic acids) extracted of the control and amended soils accounted for 7.0 to 12.9 % of the organic C of soil ($C\text{-}HS/C_{org}$). The addition of composted sewage sludge increased significantly the proportion of C_{org} associated with humic substances compared to control soil. Usman et al. (2004) explained this result by the high proportion of easily biodegradable organic components in sewage sludge. In contrast ramial chipped wood residues are formed in other high lignin concentration inducing a slower rate of decomposition of these residues compared to sludge and these amendments has no effect on proportion of humic substances

(N'dayegamiye and Angers, 1993). The proportion of the FA extracted to organic carbon of soil (C-FA/C_{org}) ranged from 3.7 to 4.4 % (Figure 2.a) and the organic amendments (RCW and CSS) added to the soil have not any effect significant on the percentage of organic carbon associated with the FA fractions. The HA fractions represent from 2.7 % to 9.1 % of the organic C of soil (C-HA/C_{org}) moreover addition of CSS to the soil increased significantly ($p<0.001$) the percentage of C_{org} associated with the HA fractions compared with the RCW and NE soils. The increase of organic carbon fraction in the form of humic acid despite the drastic procedures of extraction suggests that a part of organic carbon from organic amendments (CSS) is incorporated in humic acid fraction. The maturity and stability of organic matter are expressed by the C-HA/C-HA ratio and presented in the figure 2.b. An increase significantly of the C-HA/C-FA ratio for CSS soil ($p<0.0001$), by 3.9 fold compared to RCS and NE soils, has been observed which is not the case for RCW-type amendment. These results indicate a higher degree of humification of organic matter in the CSS soil compared to RCW and NE soils (Hsu and Lo. 2001).

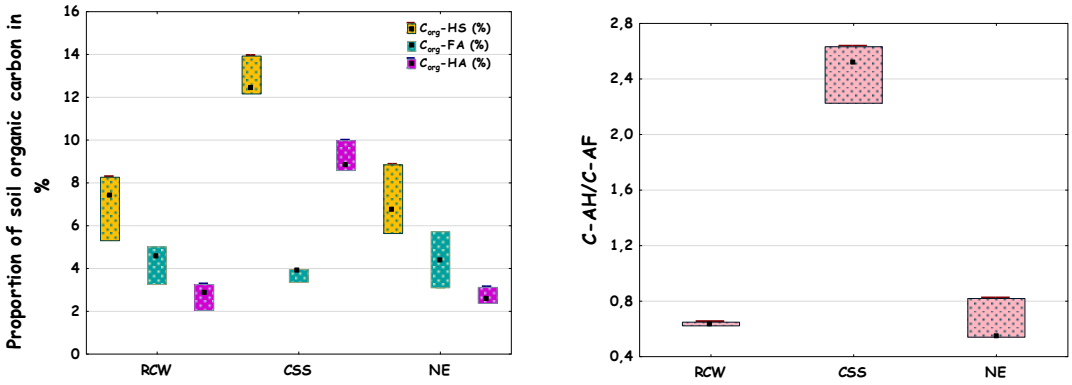

Figure 2: **a)** proportion in % of total organic carbon between HS, HA and FA; **b)** C-HA/C-FA ratio measured in the three soils. Values are: Median; Box: 25%-75%; Whisker: Min-Max (n=9).

3.4. UV–visible spectroscopy results

The absorbance ratio E_2/E_3 is considered in the literature as an indicator of the size of the organic molecules and their hydrophilicity (Thomsen et al. 2002). Addition of CSS decreased significantly the E_2/E_3 ratio ($P<0.0001$), whereas soil amended with RCW increased weakly the E_2/E_3 ratio of FA compared with soil NE respectively (Table. 3). This means that organic particle size of the RCW and NE soil was larger than CSS and the hydrophobic properties reduced in the RCW and NE soils (Guo et Chorover. 2003). Typically, the E_4/E_6 ratio is considered to be inversely related to: the particle size (Chen et al.. 1977), the molecular weights (Christl et al., 2000), the degree of condensation of the aromatic network in HS macromolecules (Senesi and Loffredo. 1999). Table 3 shows that addition of CSS to the soil increased the E_4/E_6 ratio by 30% in comparison with the NE soil whereas soil amended with RCW decreased the E_4/E_6 ratio by 19% compared with the NE soil. Our results show that CSS soil has a low degree of aromatic condensation indicating the presence of low-molecular-weight organic acids (Senesi and Loffredo, 1999. Chen et al., 1977; Christl et al., 2000; Thomsen et al., 2002). Finally, we can see that the highest E_4/E_6 ratio and the lowest E_2/E_3 ratio of fulvic acids extracted from the soil amended with CSS indicates that this soil contain more hydrophile and aliphatic compounds and less condensed and aromatic structures than those of RCW and NE soils.

Table 3: E_2/E_3. E_4/E_6 and $SUVA_{254}$ Values calculated for fulvic acid fraction by UV-Visible

	E_2/E_3	E_4/E_6
RCW (FA)	5.44 a ±0.41***	5.12 NS±1.56
CSS (FA)	2.40 ab ±0.21***	9.06 NS ±1.53
NE (FA)	4.41 b±0.67***	6.33 NS ±3.18

3.5. FTIR

FTIR has been widely used for the characterization of humic substances extracted from soils. Even if the interpretation of the analysis may be difficult due to the overlapping of spectral features of both fractions, the assignment of the absorption bands, resulting to several studies, allows us to highlight some differences once soils are amended with RCW and CSS. The FTIR spectra of HAs and FAs show typical absorption bands relative to the organic groups of the polyfonctionality of humic susbtances (Piccolo and Stevenson, 1982; Baes and Bloom, 1989; Vaca Paulin et al., 2005). The most important features are: (i) the couple of peaks of variable intensity at 2918–2926 and 2850–2855 cm^{-1} due to aliphatic C–H stretching; (ii) the strong peak centered between 1615 and 1624 cm^{-1} mostly attributed to structural vibrations of aromatic C=C and antisymmetrical stretching of COO- groups; (iii) the medium intensity absorption centered between 1398 and 1450 cm^{-1}, mostly due to the aliphatic C-H bending and symmetrical COO- stretching; and (iv) absorption at 1030 and 1090 cm^{-1} associated with C-O stretching of alcohols. The aliphatic group absorptions are often associated with weak peaks of aliphatic CH chains centered at about 770–778 cm^{-1} as both spectra show. Moreover, the weak absorption bands at 1220 and 1260 cm^{-1}, due to the C-O stretching of carboxylic groups, phenols and/or aromatic ethers appear more intense for humic substances and more particularly to a soil treated by RCW. The sharp bands at 1375 cm^{-1} present on whole spectra are attributed to residual inorganic salts resulting to an extraction process of organic matter. Moreover, the wide and large bands centered around 1100 cm^{-1} is attributed to inorganic Si-O vibrations of clay impurities co precipitated with both FAs and HAs and not eliminated by the purification process (Figure. 3)

The most significant differences in absorption bands were found for the humic substances fractions. Indeed, The FTIR spectra of treated soils with RCW and CSS show a more pronounced absorption band in 1720 cm-1 than this of the raw or standard one, indicating a larger content of carboxylic groups. The evidence that this band (shoulder around 1720 cm^{-1}) results to COOH groups is confirmed by pH values of the untreated and treated soils. The introduction of amendments decreases pH of soil and consequently increases such bands. Furthermore, the concomitant appearance of two new bands in the 1620 and 1380 cm^{-1} regions ascribed to COO^- ions resulting from the conversion of HAs to their salts confirms the last observation. The another difference feature in the spectra of HAs fractions is the appearance of weak absorption bands at 1520 cm^{-1} for amended soils, relative to N-H vibration of amide groups. The identification of such band suggests the presence of complex structural components of high molecular weight and high extents of humification.

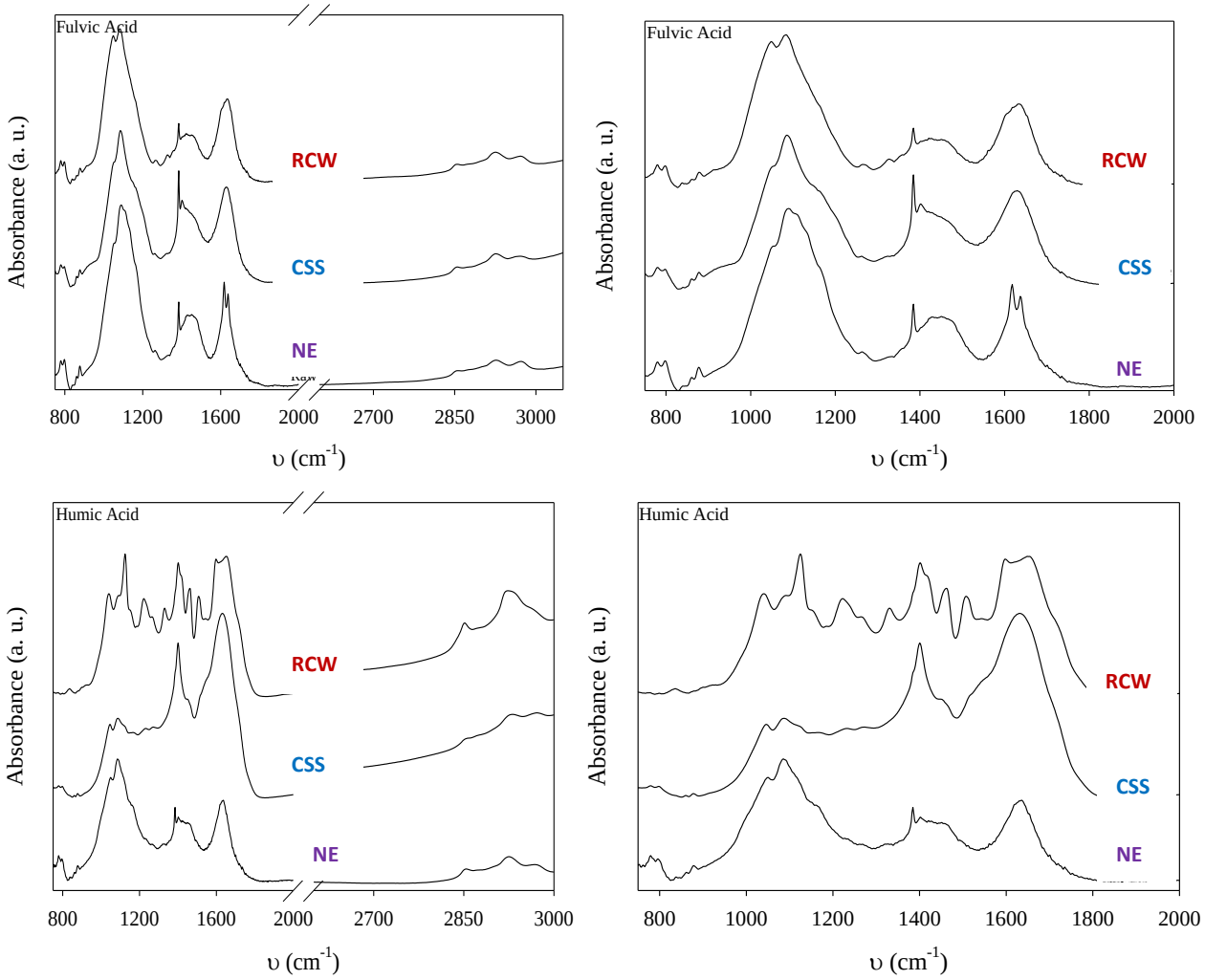

Figure 3: FTIR analysis of our treated (RCW and CSS) and untreated soils (NE).

3.6. Effect of organic amendments on dynamics of trace element

The ecological effects of trace elements, metals and metalloids, in soils are closely related to the concentration and speciation of the elements in the soil (Bruemmer et al., 1986). The average concentration values of trace elements measured in the NE soil were 1464 mg.kg^{-1} of Zn, 717 mg.kg^{-1} Pb, 620 mg.kg^{-1} of Mo, 444 mg.kg^{-1} of Cu and 80 mg.kg^{-1} of As, respectively (Table 1). Whatever the elements, their contents are largely higher than natural background (Baize, 2000), which confirms the high level of polymetallic contamination in the soils. Moreover, additions of CSS and RCW to the soil have no significant effect on the total metal concentrations compared with the NE soil.

Contents of As, Cu, Mo, Pb and Zn associated to humic substances extracted from all soils are presented in Figure 4. Cu, Pb and Zn are extracted from the fractions bound to organic constituents by NaOH in higher amounts than Mo and As. Our results show that RCW added to the soil significantly increased the concentration of extractable Zn, Pb and Cu by 4. 9 and 2 fold and slightly increased the extractable arsenic by 1.3 fold in the solution, whereas it decreased the extractable fraction of Mo by 0.8 fold compared with the NE soil. The effect of CSS added to the soil on the concentration of extractable trace elements was less than that showed by RCW, where the addition of CSS increased the extractable Zn, Pb, Mo and As by 2, 1.8, 1.2 and 1.3 fold and decreased the Cu by 1.3 fold compared with the NE soil. In general we

can note that organic amendments (RCW and CSS) added to the soil increased the extractable trace elements especially in the case of RCW amendment in comparison with the NE soil.

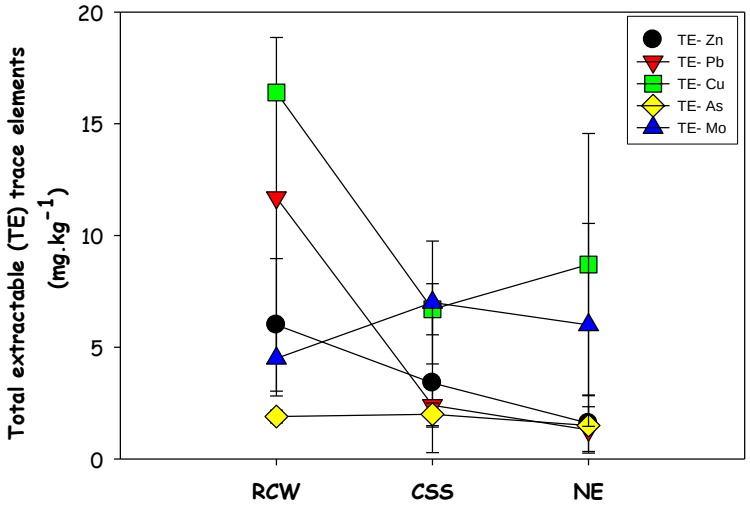

Figure 4: Total extractable Zn, Pb, Cu, As and Mo (from the NaOH extract) in mg.Kg^{-1}

Humic substances (HS) constitute a large fraction of SOM and their binding capacities affect the mobility of TE in soils. Fulvic acids are the most mobile fraction and a major component of DOM in soil (Stevenson, 1994). Therefore, the presence of soluble organic matter (as fulvic acids) may be responsible for solubilization of TE in soils whereas insoluble organic matter (as humic acids) may contribute to TE immobilization in soils. Indeed, fulvic acid is characterized by smaller molecular size fractions and high ability to form complex with trace metals more mobile than that formed with the humic acid (Stevenson, 1982). As related Hsu and Lo (2001), knowledge on distribution of TE between HA and FA fractions is essential for understanding and predicting mobility of TE in contaminated soils after organic amendments. The distribution of TE in FA and HA fractions show a large variability according to the chemical elements and the treatment (Figure 5).

Figure 5.a shows that Zn in the amended soils is more abundant in the fulvic acid fraction than in the humic acid fraction. Zn complexes by FA presented about 23 % and 32 % more than that presented by HA in the soil treated with RCW and CSS respectively. Almas et al. (2000) and Usman et al. (20004) found that addition of sewage sludge increased the solubility of Zn by formation of soluble organo-metallic complexes. Our results agree with Donisa et al., 2003, who studied the distribution of trace metals between humic and fulvic fractions in natural soils. They found that Zn associated to fulvic acid was more than that associated to the humic acid. Boruvka and Drabek (2004) also found that 95.7% of Zn bound to Fulvic acid compared to humic acid. Whereas Pb and Cu showed more than 75% from their fractions associated with HA compared with that associated with FA in treated and untreated soils, e.g. soil amended with CSS presented 80 % of Cu associated with HA compared with 20 % associated with FA (Fig. 5.b.c). He et al (1995) found also that the most Cu in the compost was recovered in HA than FA. In the case of irrigation of Mediterranean sandy soil by treated wastewater, Tarchouna Gharbi et al. (2010) demonstrated that copper had a high affinity for HA fraction. Our results don't agree with that reported by Donisa et al. (2003); Boruvka and Drabek (2004), who investigated the distribution of trace elements between the dissolved organic fraction and they found that Pb and Cu are more abundant in fulvic acid than in humic acid. Hsu and Lo (2001) isolated the substance humic from several separated swine manure (SSW) to investigate the effect of the substance humic on the leachability and availability of trace elements. They found that more than 98% of Cu and Zn complex with FA.

Arsenic is more abundant in the fulvic fraction than in the humic fraction for all soils. Arsenic associated with the fulvic acid in the soil treated with RCW, CSS and NE presented 13%, 8% and 35% more than that presented by HA respectively (Fig. 5.d). Mo is about 60% more abundant in the humic acid than fulvic acid in the soil treated with RCW and about 25% and 60% less abundant with HA than with FA in the soil treated with CSS and NE respectively (Fig. 5.e). This result can be explained by the containment of RCW soil on the quantity of organic carbon in the form of acid fulvic more than that presented by the CSS soil.

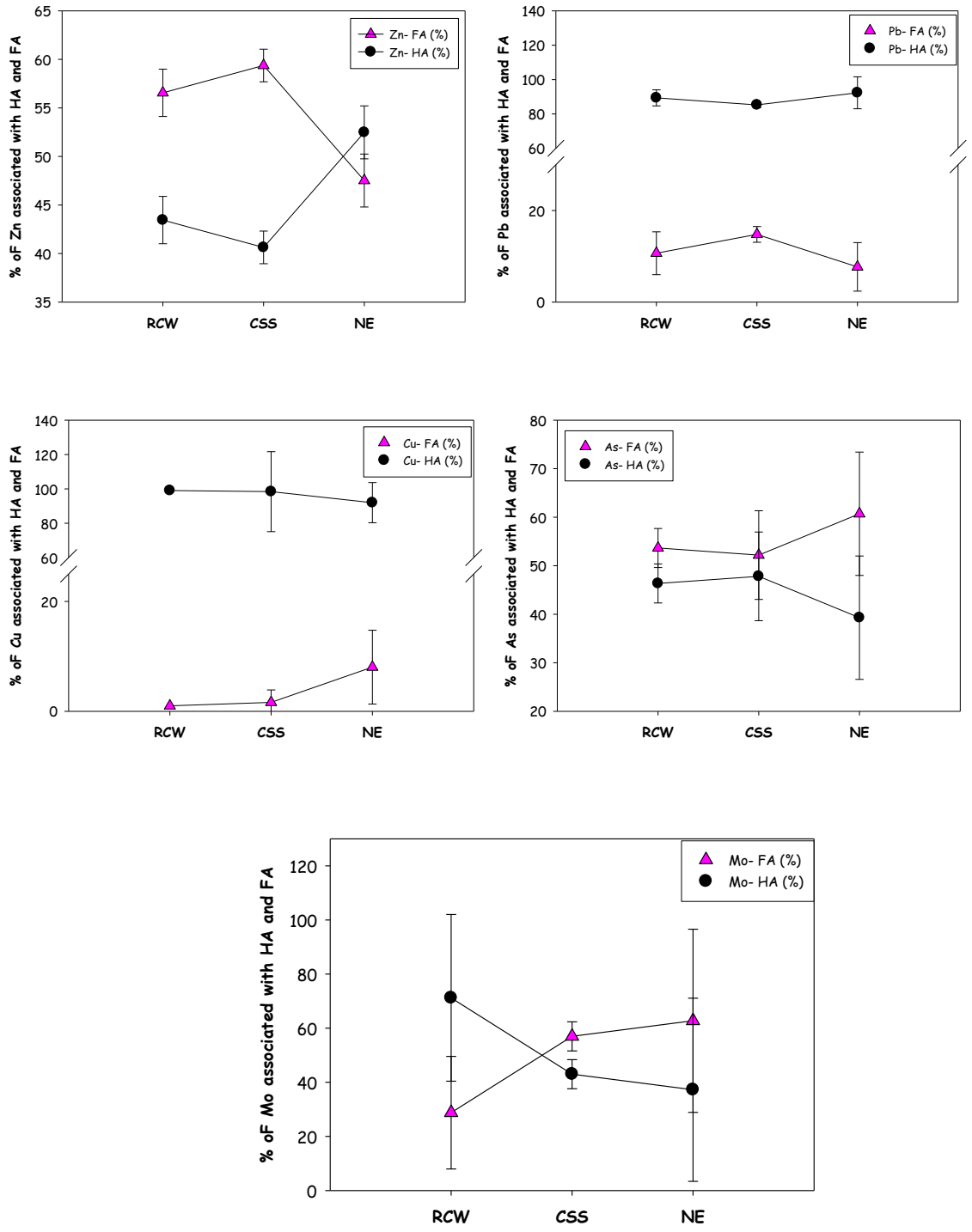

Figure 5: % of Zn, Pb, Cu, As and Mo associated with HA and FA.

4. Conclusion

The addition of RCW and CSS to the soil increased all of the total organic matter and the total organic matter in the soil. Amendments increased the HI values and OI values compared with the NE soil, especially for the soil treated with CSS which contained more aliphatic than aromatics composes. NE soil presented the lowest HI values which meaning that it was the lowest evaluation soil compared with the others soil. Organic amendments (RCW and CSS) added to the soil have not any effect significant on the percentage of organic carbon associated with the FA fractions. In contrast, the addition of CSS to the soil increased significantly ($p<0.001$) the percentage of C_{org} associated with the HA fractions compared with the RCW and NE soils. The soil amended with CSS showed the highest E_4/E_6 ratio and the lowest E_2/E_3 ratio of fulvic acids extracted from the soil amended with CSS. This indicates that this soil contains more hydrophile and aliphatic compounds and less condensed and aromatic structures than those of RCW and NE soils. Zn and As are more abundant in the fulvic acid fraction than in the humic acid fraction. In contrast Pb ,Cu and Mo were found more associated to HA compared with that associated with FA in treated and untreated soils, which can explicat the difference in the mobility and the availability of the first group (Cu and As) from the second group (Pb ,Cu and Mo).

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3éme Partie

Modélisation des résultats obtenus par les réseaux de neurones artificiels (ANN)

Au cours de cette dernière partie, nous allons présenter successivement les résultats obtenus par l'application d'un modèle appelé réseau de neurones artificiels (ANN) sur les résultats obtenus de nos travaux expérimentaux sur un site naturel. Les résultats présentés seront limités aux résultats obtenus sur le sol naturel.

Nos résultats sont présentés sous forme d'articles (acceptés ou en révision ou en préparation). Les résultats sont exposés en fonction de la méthode d'application du modèle (direct, inverse et prédiction stochastique). Les résultats présentés par la méthode directe présentent l'application du modèle de réseau de neurones pour la prédiction de la concentration de chrome dans un sol contaminé phytoremédié basé sur la prédiction des sorties en fonction d'entrées. L'application des ANN inverse présente l'application d'un modèle inverse de réseau de neurones pour l'identification des traitements de sol optimimum pour réduire la phytotoxicité du cuivre dans des sols contaminés phytorémédiés basée sur une prédiction inverse. Finalement l'application des ANN accompagné avec la simulation de Monte Carlo traite de l'analyse de l'incertitude de la concentration de cuivre phytodisponible dans un sol contaminé en cuivre phytorémédié en utilisant un réseau de neurones et des simulations de Monte Carlo.

Cette partie est divisée en trois chapitres (Chapitre 3.1, chapitre 3.2 et chapitre 3.3) et chaque chapitre présente une méthode d'application de notre modèle.

Chapitre 3.1: Application d'un modèle par réseau de neurones pour la prédiction de la phytodisponibilité du Chrome de sols contaminés phytorémédiés

Chapitre 3.2: Identification inverse des traitements optimaux pour réduire la phytotoxicité du cuivre dans des sols contaminés phytorémédiés basée sur une modélisation par réseaux de neurons

Chapitre 3.3: Effet de la variabilité des paramètres chimiques du sol sur la variabilité de la concentration du cuivre phytodisponible dans un sol contaminé phytorémédié à l'aide d'une modélisation hybride par réseaux de neurones et simulations de Monte Carlo.

Chapitre 3.1.

Application d'un modèle par réseau de neurones pour la prédiction de la phytodisponibilité du Chrome dans des sols contaminés phytorémédiés

3.1.1. Résumé de la publication

L'évaluation des concentrations de chrome dans les plantes en vue d'étudier sa phytodisponibilité nécessite la quantification d'un grand nombre de facteurs édaphiques qui influent sur sa disponibilité potentielle et sa toxicité subséquente ainsi qu'un un modèle mathématique qui prédit les concentrations relatives. En effet de nombreux facteurs du sol peuvent modifier la phytodisponibilité du chrome (Cr).

L'objectif de ce travail a été d'utiliser un nouveau modèle appelé réseau de neurones artificiels (ANN) comme un outil rapide et précis pour la prédiction de la concentration de Cr dans les haricots nains cultivés en laboratoire sur un sol contaminé comme une réponse de l'effet de plusieurs paramètres du sol tel que le pH, DOC, EC et les amendements utilisés pour remédier ces sols contaminés.

Notre site de travail a été divisé en 16 sous- parcelles ; ensuite une phytostabilisation à long terme à été appliquée sur ces 16 sous-parcelles avec quatre type d'amendements, un par sous-parcelle (plan d'expérience de type carré latin). Ces quatre types d'amendements sont: un sol non traité (UNT), un sol traité avec 0,2% de calcaire dolomitique (DL), un sol traité avec 5% de compost de fumier de volaille et d'écorce de pin (CPM) et un mélange de DL 0,2% et 5% de la CPM (DLX CPM). Finalement seize échantillons de sol (quatre répétitions) ont été échantillonnés à partir des quatre parcelles traitées avec les différents amendements (4 fois (UNT), 4 fois (DL), 4 fois (OM) et 4 fois (DLX OM)) à une profondeur de 0,25 m. Deuxièmement, une série de mesures ont été effectuées sur les 16 échantillons de sol afin de déterminer l'amendement qui limite au mieux la phytodisponibilité du chrome. Ces mesures sont les entrées de notre modèle: les 4 types d'amendement de sol, le pH du sol, la conductivité électrique du sol et le carbone organique dissous du sol. La sortie du notre modèle est la concentration de Cr dans les feuilles de haricots nains, c'est-à-dire le test de phytodisponibilité du chrome. Troisièmement, un modèle ANN a été développé et sa performance a été évaluée à l'aide d'un ensemble de données test; ce modèle a été ensuite utilisé pour prédire l'exposition des haricots nains à la concentration de Cr par rapport aux entrées du sol (paramètres chimique et les amendements).

Les résultats de cette étude montrent que les amendements sont le facteur principal qui influence la phytodisponiblité du Cr dans ce sol. Nous avons trouvé que la matière organique (CPM) est l'un des meilleurs amendements car elle réduit les concentrations de Cr dans les feuilles de haricots nains (BL) plus que les autres amendements. Le deuxième facteur qui influence la phytodisboniblité du Cr dans le sol est le pH du sol. L'effet du pH du sol a été plus net pour le sol amendé avec le CPM par rapport

aux autres sols. La conductivité électrique (EC) n'a pas eu un grand effet sur les concentrations du Cr dans les plantes, à part pour le cas du sol amendé en CPM. Nous avons trouvé aussi que l'augmentation de la concentration de COD de 14 à 34 mg. l^{-1} dans le sol a légèrement augmenté la phytodisponibilité de Cr dans les sols traités (CPM, DL, DLX CPM) et non traités (UNT).

Nos résultats ont aussi montré que cette méthode pourrait être utilisée efficacement pour étudier l'importance des effets individuels, cumulatifs et interactifs des entrées sélectionnées dans le sol sur la concentration du Cr dans les feuilles de haricots nains. Les modèles ANN développés ici ont montré qu'une stratégie pour la décontamination in situ des sols contaminés à la fois innovante et à faible coût peut être développée avec la phytostabilisation assistée par des amendements organiques et inorganiques. Il convient de noter que l'approche proposée ici (ANN) ne tient pas compte de tous les facteurs possibles qui peuvent influer sur la toxicité du sol. L'objectif principal était en effet d'illustrer le potentiel de la méthode ANN dans sa capacité de prédiction rapide et précise de la toxicité du sol.

3.2. Article 5 (*Publié dans Geochemical exploration journal*)

Application of neural network model for the prediction of chromium concentration in phytoremediated contaminated soils

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Abstract: The assessment of chromium concentrations in plants requires the quantification of a large number of soil factors that affect their potential availability and subsequent toxicity and amathematical model that predicts their relative concentrations. Many soil characteristics can change the availability of chromium (Cr) to plants in soils. However, accurate, rapid and simple predictive models of metal concentrations are still lacking in soil and plant analysis. In the present work a novel artificial neural network (ANN) model was developed as an alternative rapid and accurate tool for the prediction of Cr concentration in dwarf bean leaves grown in the laboratory on phytoremediated contaminated soils treated with different amendments. First, sixteen (4×4) soil samples were harvested from a phytoremediated contaminated site located in south-western France. Second, a series of measurements were performed on the soil samples. The inputs are the soil amendment, the soil pH, the soil electrical conductivity and the dissolved organic carbon of the soil, and the output is the concentration of Cr in the dwarf bean leaves. Third, an ANN model was developed and its performance was evaluated using a test data set and then applied to predict the exposition of the bean leaves to the Cr concentration versus the soil inputs. The performanceof the ANN method was compared with the traditional multi linear regressions method using the training and test data sets. The results of this study show that the ANN model trained on experimental measurements can be successfully applied to the rapid prediction of plant exposition to Cr.

Keywords: Artificial neural networks (ANN); Soil contamination; Chromium prediction; pH; EC; DOC

1. Introduction

Chromium in plants has received relatively little attention from researchers in comparison with other trace elements such as Cu, Cd, Ni, Al and As. Soils contaminated with Cr have serious consequences for terrestrial ecosystems, agrocultural production and human health (Adriano, 2001). Chromium has been considered as the 16th most toxic pollutant and it has become a serious health concern bcause of its carcinogenic and teratogenic effects (Kowalski, 1994).

Chromium is also considered a serious environmetal pollutant (Kotas and Stasicka, 2000), due to its widespread use in the metallurgical and chemical industries. Improper disposal, poor storage and leakage of Cr from waste discharge through the soil can release Cr to the environment, causing contamination of groundwater and adverse biological and ecological effects. Chromium is present in the environment in the form of Cr (III) and Cr (VI). These two forms show different chemical, physico-chemical and biochemical properties. Species of Cr (VI) are more soluble, more mobile, more bioavailable and more toxic than Cr (III) (Fendorf, 1995; James et al., 1997; Katz and Salem, 1994). This may be due to the fact that Cr (VI) penetrates mammalian cells more readily than Cr (III) (Wetterhahn and Hamilton, 1989). Compounds of Cr (VI) are toxic at low concentrations for both plants and animals. The presence of Cr(VI) in water, soil and wastewater has, therefore, been of great concern. Many factors can influence its migration process, such as pH (McLean and Bledsoe, 1992), dissolved organic carbon or DOC (Heijerick et al., 2003),

electrical conductivity or EC (Förstner., 1985) and soil characteristics such as cation exchange capacity, clay content and competition from other metal ions (Sauerbeck and Hein, 1991; Smith, 1994). Soluble chromates are converted to insoluble Cr(III) salts and the subsequent availability for plants decreases (Wetterhahn and Hamilton, 1989). However, soil amendments such as pH (Jackson and Miller, 2000; Kabata-Pendias and Pendias, 2000), DOC (Hsu and Lo, 2000), and EC (Hatje et al., 2003; Du Laing et al., 2009) can be used to change the soil condition and reduce the high concentration of Cr in contaminated soils (Bes and Mench, 2008; Mench and Bes, 2009). Amendments that induce solubility can be used to clean polluted sites by leaching the contaminating trace elements or by facilitating their uptake by plants (phytoextraction) (Kolbas et al., 2011).

Quantifying Cr mobility in a given soil is a critical aspect of predicting its toxicity. Since performing experimental measurements to investigate the relationship between soil parameters and Cr mobility in soil is time-consuming, difficult and expensive, the development of models simulating soil processes has increased rapidly in recent years (Minasny and McBratney, 2002). Generally two common methods are used to develop prediction models, regression methods and artificial neural networks (ANN). Several multiple linear regression (MLR) models have been developed over the past 20 years to predict the sorption of trace metals in soils (Andersen and Christensen, 1988; Elzinga et al., 1999; Reinds et al., 1995; Römkens and Salomons, 1998; Schug et al., 2000; Streck and Richter, 1997; Tiktak et al., 1998; van der Zee and van Riemsdijk, 1987). With MLR methods, the relationships between soil inputs (properties) and soil output characteristics have to be stated a priori in the regression models. An alternative to MLR is the application of ANN models where such relationships do not need to be formulated beforehand (Anagu et al., 2009; Behrens et al., 2005; Buszewski and Kowalkowski, 2006; Gandhimathi and Meenambal, 2012; Hambli, 2009; Hambli et al., 2006; McBratney et al., 2003; Minasny et al., 2004; Sarmadian and Taghizadeh Mehrjardi, 2008; Schaap and Leij, 1998).

It has been reported that ANNs provide superior predictive performance compared to conventional mathematical methods including MLR models (Andersen and Christensen, 1988; Elzinga et al., 1999; Römkens and Salomons, 1998; Sarmadian and Taghizadeh Mehrjardi, 2008; Schaap and Leij, 1998; Streck and Richter, 1997; Tiktak et al., 1998; van der Zee and van Riemsdijk, 1987). In regression models in many soil engineering situations, the input–output relationships are highly complex and are not well understood. The lack of physical understanding and of a powerful general tool for mathema ical modeling leads to either simplifying the problem or incorporating several assumptions into mathematical models. Consequently, many mathematical models fail to simulate the complex behavior of most soil engineering problems.

ANNs have been widely used in the field of soil science for prediction of soil hydraulic properties (Minasny et al., 2004; Schaap et al., 1998) generation of digital soil maps (Behrens et al., 2005; McBratney et al., 2003) and modeling of the behavior of trace metals (Anagu et al., 2009; Buszewski and Kowalkowski, 2006; Gandhimathi and Meenambal, 2012). In the cases, ANNs are trained to find model input–output relations using an iterative calibration process (training phase). Moreover, ANNs have the advantage of not imposing restrictions on inputs and outputs and can be easily applied to carry out inverse calculation (Hambli et al., 2006; Jenkins, 1997; Rafiq et al., 2001).

In the present study, a novel ANN model was developed as an alternative rapid and accurate tool for the prediction of Cr concentration in dwarf bean leaves (BL) grown in the laboratory on phytostabilized contaminated soils treated with different amendments. The inputs are soil amendment, soil pH, soil EC and soil DOC, whereas the output is Cr concentration in the dwarf BL. The performance of the ANN method was compared with a traditional MLR method using the same training and test data sets. The comparative study revealed that ANN provided a better performance than MLR method in predicting soil properties. Results showed that the neural network model led to a very rapid and accurate prediction of the soil outputs.

2. Material and methods

2.1. Site, soil sampling and soil characterization

Soil samples were collected from south western France, Gironde County (44°43′N; 0°30′W). This site has been contaminated with high concentrations of copper sulfate and chromated copper arsenate. The history of the site and the origin of these pollutants are described in detail by Mench and Bes (2009).

The study site is divided into 15 sub-sites labeled A to E and P1 to P10 depending on total topsoil Cu concentration (Fig. 1a) (Bes et al., 2010; Mench and Bes, 2009). Long-term phytostabilization experiments have been established at the P3 and P7 sub-sites by Bes (2008) and Mench and Bes (2009). Plant communities in the zone of the field trial included Agrostis capillaris, Elytrigia repens, Rumex acetosella, Portulaca oleracea, Hypericum perforatum, Hypochaeris radicata, Euphorbia ch maescyce, Echium vulgare, Agrostis stolonifera, Lotus corniculatus, Cerastium glomeratum, and Populus nigra (Bes et al., 2010). The field trial zone (150 m2) was established at site P3, which consists of two plots; that were further divided into 16 (3 m×1 m) sub-plots (Fig. 1b). On these 16 sub-plots, four different amendments were applied, one per subplot (Latin square design) and carefully mixed in the top soil (0–0.30 m) with a stainless steel spade with four replicates: untreated soil (UNT), 0.2% of dolomite limestone (DL), 5% of compost of poultry manure and pine bark (CPM), and a mixture of 0.2% DL and 5% CPM (DLX CPM). Sixteen soil samples (four replicates) were collected from the four plots treated with the different amendments (4X (UNT), 4X (DL), 4X (CPM) and 4X (DLX CPM)) to a depth of 0.25 m (Fig. 1b).

Soil pH (7.0±0.23) was measured in a 1:1 soil:water su pension using a glass electrode pH meter (Jackson, 1967). Total nitrogen (0.534 mg kg^{-1}) was determined at the INRA Laboratoire d'Analyses des sols (LAS), Arras, France using standard methods (INRA LAS, 2013). The organic matter content was determined by a modified Walkely-Blak method: 0.5 g soil was placed in a 500 ml conical flask and mixed with 10 ml of 0.17 M $K_2Cr_2O_7$, followed by addition of 20 ml of H_2SO_4, 200 ml of water, 10 ml of H_3PO_4 and 1 ml of diphenylamine indicator. Finally, the contents were titrated with 0.5 M $FeSO_4 \cdot 7H_20$. The main characteristics of site P3 (0–0.25 m soil layer) are given in Table 1. The cation exchange capacity (CEC) (3.49 cmol+/kg) was determined using cobaltihexamine chloride (Ciesielski and Sterckeman, 1997). Total metal content in P3 site was determined by ICP-AES after wet digestion with HF and HCIO4 (AFNOR ,1996; Ciesielski et al., 1997) (Table 1, column 2).

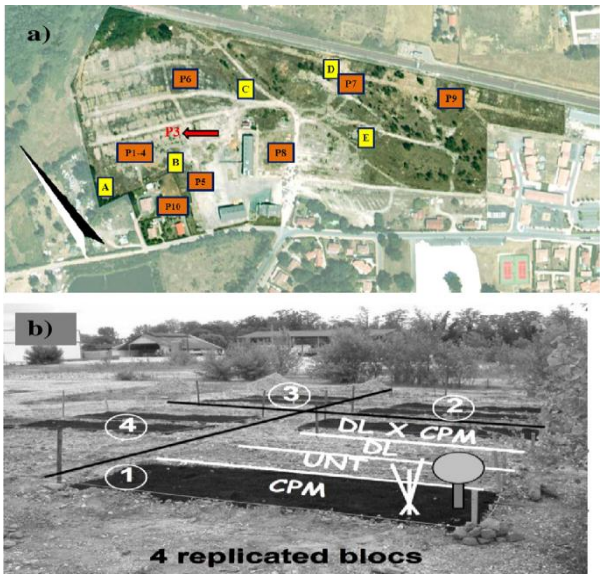

Fig. 1. a) Location of studied sites from A to E. The arrows indicate the studied site P3 (adapted from Mench and Bes, 2009). b) The two plots of soil with the different types of amendment (adapted from Bes, 2008).

Total soil metal concentrations were in the common range of French sandy soil for Cr, As and Zn, but total soil Cuwas in excess for these coarse sandy soils (Table 1, column 3). Total Cr concentrations in each of the 16 potted soil samples were measured by laser ablation inductively coupled plasma-mass spectrometry (LA-ICP-MS). Prior to metal content measurements, the accuracy of the metals determination was checked by performing calibrations with a homogeneous combination of certified reference materials such as NIST-610 (artificial glass), GOR-128-G (komatiite natural glass) and NIST-361 (steel). Little difference was found between the measured and the reference results. In our case, the LA-ICP-MS measurements produced an averaged error in the range of −1.2% to

1.17%. After the calibration phase, 4 repeated measurements were performed for each soil generating 64 testing results (4 soils×4 amendments×4 replicates). The precision of the metal co tent measurement was assessed by the standard deviation (SD) and the coefficient of variation (COV) (Table 2).

2.2. Germination tests

A phyto-toxicological test was applied to the 16 soil samples to assess whether the use of amendments on the field trial zone, assisted by phytostabilisation, is able to reduce the concentrations of bioavailable fractions of Cr for plants. To achieve this objective, one kilo of each soil (4X (UNT), 4X (DL), 4X (CPM), 4X (DLX CPM)) was placed in a pot after sieving (2 mm). Then, four seeds of dwarf beans (Phaseolus vulgaris) were sowed in each potted soil and cultivated for 18 days in controlled conditions (16 h light/8 h darkness). Soil moisture was maintained at around 50% of the field water capacity with additions of deionized water after weighing. Then, the soil moisture was raised to 80% (11– 13% of air-dried soil mass) at the beginning of the germination stage of the grains. At the end of the growing period, the plants were harvested and then the dryweights of roots, shoots, and primary leaves were dete mined after drying at 70 °C. The dwarf beans were weighed (35–150 mg) directly into Savillex polytetrafluoroethylene PTFE 50 ml vessels. To each vessel 2 ml H_2O and 2 ml supra-pure 14 M HNO_3 were added and the vessels were heated open at 65 °C for 2 h. Then, the vessel caps were closed and the containers were left overnight at 65 °C (12–14 h). After that they were opened, 0.5 ml of H_2O_2 (30%) was added to each sample and left at 75 °C open for 3 h. Then 1.5 ± 0.5 ml of fluorhydric acid HF (48%) was added to each sample, vessel caps were closed and the samples were left at 100 °C overnight. Then, vessels were opened and kept at 120 °C for 4–5 h evaporating to dryness, taken off heat, 1 ml HNO_3+5 ml H_2O+0.1 ml H_2O_2 was added to each vessel, gently warmed up and after cooling down made up to 50 ml.

Trace element concentrations in digests were determined by ICP-MS (Varian 810- MS) using standard solution of measured trace elements, in our case, Reference Standard Solution of Chromium (1000 ppm±1%/ Certified) was used. The accuracy of the metals determination was checked by performing calibrations with standard reference solution. Strong correlation was found between the measured and the reference results (R^2=0.998). After the cal bration phase, 4 repeated measurements were performed for each digest generating 64 testing results (4 repeated×16 digests). The precision of the metal content measurement was assessed by the standard deviation (SD) (Table 4), then the Cr concentration in a given dry weight, expressed here, the mineral masses (g per pot) were determined by multilying Cr concentration in the BL (mg kg^{-1}) in dry weight of BL (g DW) (Table 4).

Table 1: Main characteristics of site P3 (0–0.25 m soil layer).

Parameters	Site P3	Background values in French sandy soils[a]
Sand %	83.5 ± 1.1	
Silt %	11.5 ± 0.9	
Clay %	3.8 ± 1.2	
C/N	17.2	
OM (g.kg^{-1})	15.9	
CEC (cmol^{+}.kg^{-1})	3.49	
Organic C (g.kg^{-1})	9.19	
total N (g. kg^{-1})	0.534	
pH	7.0 ± 0.23	
As (mg.kg^{-1})	9.8	1.0-25[b]
Co (mg.kg^{-1})	2	1.4-6.8
Cu (mg.kg^{-1})	674 ± 126	3.2-4.8
Cr (mg.kg^{-1})	23	14.1-40.2
Mn (mg.kg^{-1})	181	72-376
Ni (mg.kg^{-1})	5	4.2-14.5
Zn (mg.kg^{-1})	46	17-48

[a] Median and high vibrissae values except for As (Baize, 1997; Baize and Tercé, 2002). [b] Common As mean values for all French soil types (Baize and Tercé, 2002).

2.3. Characterization of soil solution

After harvesting the dwarf beans, the soils were watered with distilled water, and maintained daily at 80% of field capacity (11–13% of air-dried soil mass) for 15 days. After 15 days, three Rhizon soil-moisture samplers from Rhizosphere Research Products (Wageningen, Holland) were inserted for 24 h with a 45° angle into each potted soil (4×16 soil) to collect (30ml) of soil solution from each pot. DOC was analyzed in the solution of soils by a Shimadzu© TOC 5000A analyzer. Before DOC measurements, the accuracy of the analyzer was checked by performing calibration with standard reference solution (potassium hydrogen phthalate (KHP) (1000 mg C/L)). In our case, the measurements produced an R^2= 0.999. After the calibration, 4 repeated measurements of DOC were performed for each soil solution. The precision of the DOC measurement was assessed by the SD and the COV (Table 2). Soil pH and EC were determined in the same soil solutions by pH meter and the EC meter (Table 2).

Measured soil variables included soil pH, soil EC, DOC and Cr concentration in the BL with 4 replications for each measurement (4×4 measurements) for the ANN training. Then, a neural network model was developed and trained to predict the exposition of the BL to the high Cr concentration in the soil. The inputs are the soil amendment, soil pH, soil EC and DOC. The output is Cr concentration in the BL.

2.4. Selection of soil factors

The mobility of trace elements plays a major role in the behavior and transfer of metals in soils. Mobility, availabi ity and/or bioavailability of metals is controlled by several factors such as the soil's chemical and physical properties (pH, EC, DOC, temperature, ion charges, etc.), plant spcies and their related factors (Davies, 1992; Kerndorff, and Schnitzer, 1980; Sauerbeck and Hein, 1991; Soon and Bates, 1982; Vogeler et al., 2001;Weng et al., 2002). It has been reported that the relationships between the mobility of metal concentrations in plants and a given soil are mainly influenced by the soil properties related to the ion charges Fischer, 2000; Kerndorff and Schnitzer, 1980; Vogeler et al., 2001; Weng et al., 2002). Therefore, in the current preliminary study, the soil inputs for the ANN model were limited to the three measurable factors considered to be the most influential (pH, DOC and EC) on the mobility and availability of metals in the soil.

Jackson and Miller (2000) and Kabata-Pendias and Pendias (2000) reported that the addition of amendments to soil increased the soil pH compared to the untreated soil, leading to a reduction in the mobility and the availability of metals to plants. The second variable used in our work was DOC. Hsu and Lo (2000) noted that the addition of amendment to the soil increased the soil DOC compared to that of untreated soil, which led to increasing plant mass; reducing the bioavail bility of metals by forming ligands with dissolved organic matter. The third parameter was EC. Du Laing et al. (2008, 2009) and Hatje et al. (2003) found that an increase in conductivity (salinity) led to increasing the mobility of some metals such as Cd and Zn, thereby increasing the availability and bioavailability of these metals to plants.

To prepare the training data for the ANN, 64 different measurements (4 soils×4 amendments×4 replicates) were performed on the four different soils subjected to four different treatments (UNT, DL, CPM and DLX CPM). Because of the noise (uncertainty) concerning the inputs measurement which can be characterized by a given a set of scattered input data, the 64 experiments were used for the training phase instead of 16 (the total number of experimental replicates) to ensure a more robust processing of the scattered measurements (Table 2). The 64 experiments were used for the training phase instead of 16 (the total number of experimental replicates) to ensure a more robust processing of the scattered measurements. Presenting the entire patterns (64) ensured the reduction of the ANN prediction sensitivity to noise related to inputs (Hambli, 2009; Kafadar, 1994; Velleman, 1980). The

inputs and their minimum/maximum levels are given in Table 3.

Table 2. The experimental values (pH, EC, DOC and Cr). Four replications were performed for each measurement generating 64 testing results (4 soils × 4 amendments× 4 replicates) allowing for the determination of the mean values, standard deviations (SD) and coefficient of variations (COV).

Soil reference	Amendments	Mean pH	SD-COV (%)	Mean EC (µS.cm^{-1})	SD-COV (%)	Mean DOC (mg.l^{-1})	SD-COV (%)	Mean Cr concentration in the soil (mg.kg^{-1})	SD-COV (%)
B1-1	UNT	7.12 NS		1114 NS		15.93*		15.97**	
B2-8	UNT	7.31 NS		1230 NS	214.7-18.31%	14.34*		16.21**	
B3-15	UNT	7.28 NS	0.15-2.08%	917 NS		21.74*	4.01-7.75%	15.81**	0.98-6.30%
B4-10	UNT	6.99 NS		1430 NS		24.32*		14.07**	
B1-2	CPM	6.98 NS		1018 NS		27.08*		14.67**	
B2-5	CPM	7.60 NS		884 NS		20.64*		15.03**	
B3-16	CPM	7.46 NS	0.27-3.67%	771 NS	122-14.24%	25.85*	4.50-5.44%	15.15**	0.34-2.31%
B4-11	CPM	7.26 NS		754 NS		33.99*		14.40**	
B1-4	DL	7.49 NS		1654 NS		26.34*		17.16**	
B2-7	DL	7.67 NS		1000 NS	308.3-24.79%	25.76*		16.98**	
B3-14	DL	7.43 NS	0.14-1.87%	1013 NS		26.52*	4.14-6.96%	17.13**	0.11-0.66%
B4-9	DL	7.34 NS		1308 NS		38.46*		16.93**	
B1-3	DLX CPM	7.45 NS		1192 NS		33.12*		16.09**	
B2-6	DLX CPM	7.00 NS		1347 NS	152.2-11.90%	34.26*		15.91**	
B3-13	DLX CPM	7.07 NS	0.32-4.46%	1457 NS		29.16*	2.50-7.02%	15.93**	0.2-1.27%
B4-12	DLX CPM	7.59 NS		1119 NS		29.75*		16.35**	

* Significant for p<0.05, ** highly significant for p<0.001, NS (non significant)

2.5. Statistical analysis

The total soil solution concentrations of Cr, pH, DOC, EC, foliar elemental concentrations, foliar mineral masses of elements and leaf dry weight (DW) yields were statistically analyzed by ANOVA (Statistica) to evaluate the influence of the treatment and the different effects of soil variables on each other. All analytical determinations were pe formed in four replicates. Differences were considered statistically significant at p**<**0.05.

2.6. Artificial neural network

The ANN architecture is composed of an input layer, a certain number of hidden layers and an output layer in forward connections. Each neuron in the input layer represents a single input parameter. These values are directly transmitted to the subsequent neurons of the hidden layers. The neurons of the last layer represent the ANN outputs (Fig. 2). The output yi^{m} of neuron *i* in a layer *m* is calculated by (Hambli et al., 2006; Jenkins, 1997; Rafiq et al., 2001):

$$y_i^m = f\left(v_i^m\right) \qquad (1)$$

$$v_i^m = \sum_{j=1}^{L} w_{ji}^{m-1}\, y_j^{m-1} + b_i^m \qquad (2)$$

Where y_i^0 are the model inputs, v_i^m are the outputs of the layer *m*, *f* is the activation function, *L* is the number of co nections to the previous layer, w_{ji}^{m-1} corresponds to the weights of each connection and b_i^m is the bias, which represents the constant part in the activation function.

Among activation functions, the sigmoid (logistic) function is the one most usually employed in ANN applications. It is given by (Hambli et al., 2006; Jenkins, 1997; Rafiq et al., 2001):

$$f\left(v_i^m\right) = \frac{1}{1+\exp\left(-\theta v_i^m\right)} \qquad (3)$$

where θ is a parameter defining the slope of the function (θ=0.9).

The training process in ANNs involves presenting a set of examples (input patterns) with known outputs (target output) (Hambli, 2009; Hambli et al., 2006; Jenkins, 1997; Rafiq et al., 2001). The system adjusts the w_{ji}^{m-1} of the internal connections to minimize errors between the network output and target output (Levenberg–Marquardt back-propagation or BP training algorithm in the current

work). The knowledge is represented and stored by the strength (weights) of the connections between the neurons.

In the present work, an in-house ANN program called Ne romod written in Fortran (Hambli, 2009; Hambli et al., 2006) was applied. The basic ANN configuration employed in this study has a double hidden layer with six neurons of each layer with a learning rate factor η=0.1 and momentum coefficient α=0.1 (Fig. 2). The learning rate coefficient η and the momentum term α are two user-defined BP algorithm training parameters that affect the learning procedure of the ANN. The training is sensitive to the choice of these net parameters. The learning rate coefficient, employed during the adjustment of w_{ji}^{m-1}, was used to speed up or slowdown the learning process. A larger learning coeffcient increases the weight changes, hence large steps are taken towards the global minimum of error level, while smaller learning coefficients increase the number of steps taken to reach the desired error level.

Tests performed for more than two hidden layers and different η and α parameters showed no significant improvement in the obtained results. A total of 64 measurements were performed. The proportion of ANN training set from the available data ranged generally from 25% to 80%.

The proportion of ANN testing set from the available data is about 15 to 20% and the proportion of the validation data set is about (5 to 15%) (Haykin, 2009). In the current work, the experimental data (64 cases) were subdivided in three sets: 62.5% of the data were used for training (40), 25% for testing (16) and 12.5% for validation (8) covering a wide range of the experiments for validation (2 samples from every experiment variant). The testing data were not used for training. The testing data provided cross validation during the ANN training for verification of the network prediction accuracy. The validation data were used to measure the performance of the predictive capability of the ANN after complete training.

In order to avoid data saturation and artifacts related mainly to the coding of the amendments (non-numeric data), the input and output variables were normalized between 0 and 1 using:

$$x_i^{norm} = \frac{x_i - x_i^{\min}}{x_i^{\max} - x_i^{\min}} \quad (4)$$

where xi, xi min, xi max and xi norm denote, respectively, values of input (output) variables i, minimum value of input (output) variable, maximum value of input (output) variable and the normalized value of i.

The de-normalized value of the ANN was computed using:

$$y_t = y_{\min} + y_{norm}\left(y_{\max} - y_{\min}\right) \quad (5)$$

where y_t, y_{min}, y_{max} and y_{norm} are, respectively, real valued output variable, minimum and maximum values of real-valued output and the normalized output value from the neural-ANN model.

Table 3. Selected inputs and output for ANN training (UNT=#1, CPM=#2, DL=#3, DLX CPM=#4)

Inputs	**Level**	**Min value**	**Max value**
Soil treatment	4	#1	#4
pH	4	6.98	7.6
EC (µS.cm^{-1})	4	754	1654
DOC (mg.l^{-1})	4	14.337	34.260
Output			
Cr concentration in the bean leaves (mg.kg^{-1})			

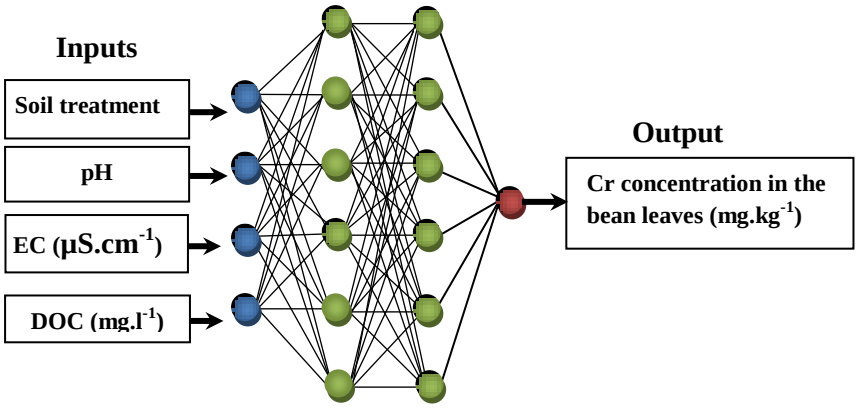

Figure 2. Neural network architecture composed of four input layers, two hidden layers and one output layer.

2.6.1. Coding of the amendments

The amendments are model independent inputs, therefore, they can be represented using sequential codes (integer) as a non-numeric indexes from 1 to 4 (UNT=#1, CPM=#2, DL=#3, DLX CPM=#4). Such non-numeric data encoding is common when applying ANN models (Haykin, 2009). An alternative approach is to use the one-of-N encoding technique, which consists of transforming a many-state variable (four states in our case) into several binary v riables (one for each state) to represent a single nominal variable. The number of numeric variables equals the number of possible values; one of the N variables is set to one, and the others are set to zero. In the current case, the input variable "amendment" was replaced by 4 binary variables, (e.g., UNT=1000, DL=0100, CPM=0010, DLX CPM=0001; where 0 denotes to inactive and 1 active Ruggiero, 1997).

In certain cases, a nominal variable with a large number of states would require a prohibitive number of numeric variables for one-of-N encoding, which can increase considerably the ANN size and making the training difficult. If such is the case, it is possible to model the nominal variable using a single numeric sequential encoding if these non numeric data are inputs. These sequential codes are not quantities or values related to the treatments or any classification of them. In the current ANN model, these codes are treated by the ANN algorithm in the following general form:

○ In case #1 (UNT amendment), the prediction model is determined by the ANN learning phase related to the case 1 representing the UNT treatment.

○ In case #2 (CPM amendment), the predictionmodel is determined by the ANN learning phase related to the case 2 representing the CPM treatment.

○ In case #3 (DL amendment), the prediction model is determined by the ANN learning phase related to the case 3 representing the DL treatment.

○ In case #4 (DLX CPM amendment), the prediction model is determined by the ANN learning phase related to the case 4 representing the DLX CPM treatment.

In addition, during the training phase the assigned codes for the amendments [1, 4] are converted in the normalized range [0, 1] using Eq. (4) to avoid coding artificial effects and de-normalized after prediction in the range of [1, 4] using Eq. (5).

2.7. Multiple linear regression models

The most common method used to quantify relationships between inputs and outputs is to employ multiple linear regressions in the form:

$$Y_i = \alpha_i + \sum_{j=1}^{n} \beta_i \, X_i \qquad (6)$$

where Yi is the output variable i, Xi are the input variables related to the sample, i, αi and βi are the model regression coefficients determined by a fitting procedure using generally least squares method.

3. Results and discussion

3.1. Experimental results

3.1.1. Soil solution characteristics

Table 2 shows the physico-chemical parameters (i.e., pH, DOC and EC) of the 16 soil solutions and the total soil Cr concentration measured in the 16 contaminated soils. These measurements concerned the 16 pots containing the 16 types of soils: 4X UNT, 4X DL, 4X CPM, and 4X DLX CPM. Amendment incorporation into the soil increased soil pH slightly in the treated soil compared to the untreated one. This effect was in decreasing order based on mean values from 4 replicates: DL (7.5±0.14)>CPM (7.4±0.27)>DLX CPM (7.3±0.29)>UNT (7.2± 0.15). The organic matter added to the soil (i.e., CPM) resulted in reduction of EC (856.75±122.00 μS cm^{-1}) compared to the DL (1243.75± 308.27 μS. cm^{-1}), DLX CPM (1278.75±152.18 μS. cm^{-1}) and the UNT (1172.75±214.72 μS. cm^{-1}).

The soil amendments had no significant effect on the pH and EC of the four types of soil. In contrast, the addition of amendments significantly increased the DOC in the soil solution compared to the untreated soil (p=0.019). This effect was in the following order: DLX CPM (34.6±2.50 mg. l^{-1})>DL (29.2±6.14 mg. l^{-1})>CPM (26.8±5.49 mg. l^{-1})>UNT (19.0± 4.72 mg. l^{-1}). The amendments also had a significant effect on the total Cr concentration in the soils (p=0.000501). A good correlation was observed between soil pH and DOC (R^2=0.589), but poorer or lack of correlations were observed between the other variables used in our experiment.

3.1.2. Concentration and mineral mass of Cr in the bean leaves

Table 4 shows that the measured Cr concentrations in the BL were less than the toxic concentrations measured in the tops of plants reported by Davis et al. (1978), Gough et al. (1979) and Kitagishi and Yamane (1981). The mean value of foliar Cr concentrations in the beans decreased from 1.438±1.23 in the UNT soil to 0.733±0.08 in the soil amended with CPM, to 0.588±0.17 in the soil amended with DL and to 0.652±0.012 in the soil amended with DLX CPM. However, the soil amendments (CPM, DL, DLX CPM) had no significant effects on the Cr concentrations in the BL (p=0.436) (Table 4).

Leaf Cr mineral mass (g ha^{-1}) was computed with foliar Cr concentrations (mg kg^{-1} DW) and foliar biomass (g DWper pot). We can clearly note in Table 4 that soil amendments decreased the mineral mass of Cr in the BL compared to the UNT plants (0.094±0.03 g per pot). The lowest Cr mineral masses were observed for the DLX CPM plants (0.067±0.01 g per pot). This difference in the mineral masses is notably driven by the lower foliar Cr concentrations and dry weight in the DLX CPM plants. The DL and DLX CPM additions decreased the Cr mineral masses more than those for CPM and UNT. While amendments added to soil had a significant effect on the total Cr concentration (p=0.000501) in the four soils UNT (15.52 mg. kg^{-1}), CPM (14.81 mg. kg^{-1}), DL (17.05 mg. kg^{-1}) and DLX CPM (16.07 mg. kg^{-1}), there was no significant effect of soil amendments on the concentration and the mineral masses of Cr in the plants.

Table 4: Dry weight (g), concentration (mg kg−1) and mineral mass of Cr (g per pot) measured in the BL.

Soil amendments	Dry Weight (g)	Cr concentration in the plant (mg. kg^{-1})	Mineral mass (g per pot)
UNT	0.087±0.04	1.438±1.23	0.094±0.03
CPM	0.112±0.02	0.733±0.08	0.083±0.01
DL	0.122±0.02	0.588±0.17	0.069±0.00
DLX CPM	0.103±0.00	0.652±0.012	0.067±0.01

3.2. Neural network modeling results

The ANN was trained with 3×104 epochs (number of training cycles). The training performance was assessed by the root mean square error (RMSE). At the end of the training phase, the RMSE convergence value was 1×10^{-5}. Table 5 shows the experimental and ANN predicted values of Cr concentration in the BL for the four soil groups (four diferent amendments) and their corresponding correlation coefficients.

An average correlation coefficient of 0.9998 between the measured and ANN predicted values as found. This indicates that the ANN model was able to predict the Cr concentration in the BL rapidly and accurately. In order to investigate the effects of the soil amendments, soil pH, soil EC and DOC inputs on the Cr concentration in the BL, a parametric study of the ANN output with respect to the inputs was performed for each soil treatment with the trained ANN.

For each input factor, four values in the interval between the minimum and maximum values of each input were applied here to capture the non-linear response (Table 6). Full factorial design generated 256 (4×4×4×4) combinations to study the effect of each input on the Cr response. The computation time was about 2 s.

To explore the relationships between the inputs and the output, several response surfaces (RS) were plotted consising of 3D graphical representation of a response between two independent variables (inputs) and the output. The 3D RS plots provide insight into the behavior of the system and enable investigation of the parametric Cr concentration results versus the levels of the effecting factors predicted by the ANN model. Fig. 3 shows the coupled effects of the pH and EC on the Cr concentration in BL for two different values of DOC (14 and 34 mg; l^{-1}).

The predicated Cr concentration depends strongly on the soil factors and the type of amendments. It can be seen in the left-hand column of Fig. 3 that soil amendments (CPM, DL and DLX CPM) and other factors such as pH, EC and low DOC (DOC=14 mg. l^{-1}) decreased the Cr concentration in the BL to 1, 1.2 and 1.4 mg. kg^{-1}, respectively, compared to the untreated soil which presents a reference Cr concentration of about 2.4 mg. kg^{-1}. The same effect of soil amendments can be observed when the DOC value increased to 34 mg. l^{-1} (Fig. 3 right-hand column). If we compare the right and left columns of Fig. 3, we can see that the increase in DOC concentration from 14 to 34 mg. l^{-1} in the soil slightly increased the availability of Cr to plants in the treated (CPM, DL, DLX CPM) and UNT soils by 0.3, 0.2, 0.2 and 0.4 mg. kg^{-1}, respectively.

Barcelona and Holm (1991), Pantsar-Kallio et al. (2001) and Seaman et al. (2001) reported that oxidation and reduction reactions are important in the fate, transport, and toxicity of Cr in the soil. These reactions are governed by many factors including organic matter, pH, aeration, soil moisture content, wetting and drying, microbial activity, the content in clay minerals and availability of electron donors and acceptors. Moreover, the amendments added to the soil have the capacity to change the Cr from Cr(VI),

which has a high mobility and toxicity and facilitates leaching, to Cr(III), which is relatively insoluble and resistant to leaching (James et al., 1997). Fendorf (1995) also reported that the reduction of Cr in soils is accelerated by the presence of organic matter and divalent iron. If Cr in the soil is converted to trivalent oxide or co-precipitated with Fe hydrous oxide or organic matter, it is likely to remain stable for a long time. Our results agree with those of Hsu et al. (2000), who reported that the high affinity of metals for CPM had two-sided consequences. The first is that in the presence of DOC the mobility of trace elements increases by forming complexes with the humic acid and fulvic acid, leading to the high mobility and availability of metals to plants. Secondly, non soluble high molecular weight organic acids can retain significant concentrations of trace metals in soil upon soil acidification, thus decreasing their mobility (Chirenje et al., 1999).

The concurrent effect of the three studied factors can be observed clearly in the soil treated with CPM (Fig. 3- right-hand column), where it can be seen that the increase in the DOC value (34 $mg.l^{-1}$) led to an increase in the Cr availability to the plant from 1.2-1.4 $mg.kg^{-1}$ in the case of DOC=34 $mg.l^{-1}$ compared to soil treated with CPM containing less DOC (14 $mg.l^{-1}$) where it decreased from 1.2 to 0.8 $mg.kg^{-1}$. The increase in pH leads to a slight decrease in Cr concentration in plants, whereas the decrease in EC leads to a strong increase in Cr concentration in the BL from 0.2 at EC=1100 $mg.kg^{-1}$ to 1.3 at EC=800 $mg.kg^{-1}$ in the case of DOC=34 $mg.l^{-1}$. This result can be explained by the fact that in the case of anions (Cr (VI)), the decrease in pH promotes the release of HO-ions. This then leads to a decrease in competition between anions and HO-ions, which then accelerates the formation of new phases. Thus, the solubility of Cr anions decreases when pH decreases. Dzombak and Morel (1990) reported that when soil pH increases, the mobility of metals decreases, thereby decreasing the uptake of Cr by living species as in the case of soil treated with DL, CPM at DOC=14 $mg.l^{-1}$. Another important factor that has a major effect on the bioavailability of Cr is EC. Increases in chloride, sulfate, carbonate, bicarbonate, nitrate and phosphate concentrations in solution lead to the formation of metallic salts, which increase the EC of the soil solution. An increase in salinity is associated with an increase in the concentration of major elements (Na, K, Ca, Mg), which compete with trace metals for sorption sites (Tam and Wong, 1999). This is different from our results where we found that the increase in DOC and pH and decrease in EC lead to an increase in Cr concentration in plants.

The predicted findings suggest that due to the large variation of Cr concentration in the BL with different treatments, the best amendment for reducing soil Cr toxicity is the (CPM) treatment. This reduced Cr concentration from 2.4 $mg.kg^{-1}$ in the untreated soil to 1 $mg.kg^{-1}$ (case of DOC=14 $mg.l^{-1}$) and from 2.8 $mg.kg^{-1}$ in the untreated soil to 1.2 $mg.kg^{-1}$ (case of DOC=34 $mg.l^{-1}$). These two DOC values result in non-linear Cr variation versus pH and EC. However, the DL and DLX CPM treatments generated intermediate Cr concentration levels but with a more stable (linear) variation compared to that of the CPM treatment.

When all the results are considered, it can be seen that the Cr tends to evolve from non-linear responses for UNT and CPM soils to linear responses for DL and DLX CPM soils.

UNT soil generated the highest non-linear variation in Cr concentration, suggesting that the results are sensitive to pH and EC. Such a variation is reduced, however, with the addition of CPM and DL or with a combined treatment (DLX CPM). We found that among the investigated factors, pH and DOC are the most important parameters affecting the Cr concentration in BL.

However, there are only a limited number of published studies available for describing the techniques of stabilization of Cr in the soil. Because of the low reactivity of its common species in soils, even if Cr is added to soil in the mobile form Cr(VI), it can be converted to its stable form Cr(III). Jop et al. (1987) reported that the dominant species of Cr(V1) at pH > 6.5 is chromium oxide (the case of our soil). As Cr(VI) species are negatively charged, they do not complex with anionic particulate matter and are more bioavailable. In our experience, we have noted that the addition of both organic and inorganic amendments to the soil can decreased the bioavailability of Cr with reference to untreated soil.

Table 5: Comparison between averaged measured and ANN predicted values of Cr concentration in the bean leaves for the four soil groups. The determination coefficient R2 is based on the eight experimental samples kept for validation.

Soil (UNT)		**Soil (CPM)**		**Soil (DL)**		**Soil (DLX CPM)**	
Measured	**NN**	**Measured**	**NN**	**Measured**	**NN**	**Measured**	**NN**
3.29	3.285	0.762	0.764	0.519	0.519	0.524	0.523
0.748	0.746	0.709	0.710	0.775	0.773	0.819	0.820
0.601	0.600	0.392	0.391	0.892	0.890	0.805	0.804
0.661	0.662	0.498	0.496	0.618	0.616	0.781	0.780
R^2=0.9998		**R^2=0.9986**		**R^2=0.9989**		**R^2=0.9991**	

Table 6. Selected four inputs for the ANN prediction. Full factorial combinations generate 256 ($4 \times 4 \times 4 \times 4$) data to investigate their effects on soil response.

Inputs	**min**	**max**	**Level 1**	**Level 2**	**Level 3**	**Level 4**
Soil treatment	#1	#4	UNT	CPM	DL	DLX CPM
pH	6.98	7.6	7.0	7.2	7.4	7.6
EC ($\mu S.cm^{-1}$)	754	1654	800	1100	1400	1600
DOC ($mg.l^{-1}$)	14.337	34.260	14	20	28	34

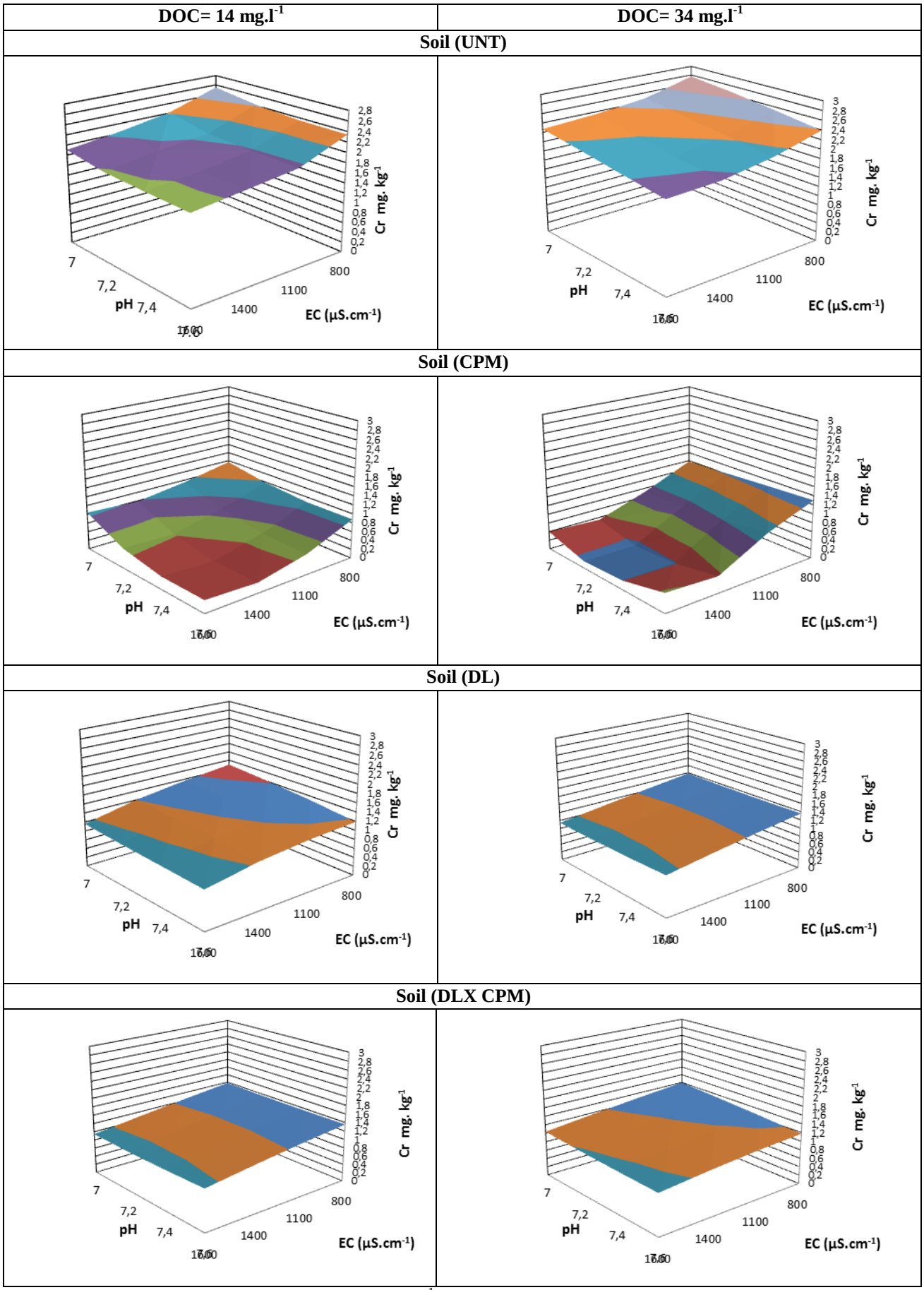

Figure 3. Chromium concentration in BL in mg.kg^{-1}: effects of soil pH and EC for two different limit values of DOC

3.3. Comparison of MLR and ANN performance

Four MLR experiments were performed, one each of the four soil training data pertaining to every amendment, to quantify the relationships between Cr concentration in BL and the soil factors (pH, EC and DOC). The software used for the purpose of statistical analyses was Statistica 10. These four MLR equations are:

Amendment	MLR equations per amendment
UNT	$Y_{BL} = 2.255 + 0.0623 \times x_{pH} - 6.44e^{-4} \times x_{EC} + 0.00128 \times x_{DOC}$
DL	$Y_{BL} = 2.987 - 0.113 \times x_{pH} - 1.26e^{-3} \times x_{EC} + 0.00097 \times x_{DOC}$
CPM	$Y_{BL} = 1.851 + 0.0124 \times x_{pH} - 6.67e^{-4} \times x_{EC} + 0.00115 \times x_{DOC}$
DLX CPM	$Y_{BL} = 1.986 + 0.0118 \times x_{pH} - 6.61e^{-4} \times x_{EC} + 0.00101 \times x_{DOC}$

where x_{pH}, x_{EC} and x_{DOC} denote, respectively, values of pH, EC and DOC.

After determining of these equations, comparison between predictive results of the ANN and MLR models was performed for the training and test data sets. The obtained coefficients of correlations among the results (Table 7). This indicates that the results of ANN model are better than that of MLR models for all amendment cases. Our finding agrees well with those of van der Zee and van Riemsdijk (1987), Andersen and Christensen (1988), Streck and Richter (1997), Römkens and Salomons (1998), Tiktak et al. (1998), Schaap and Lei (1998), Elzinga et al. (1999), Sarmadian and Taghizadeh Mehrjardi (2008).

Table 7. Correlation coefficients obtained by ANN and MLR methods.

Method	UNT		CPM		DL		DLX CPM	
	Training data	Training data	Training data	Training data	Training data	Training data	Training data	Training data
Neural network	0.999	0.998	0.998	0.996	0.998	0.997	0.999	0.995
Linear regression	0.876	0.695	0.792	0.702	0.765	0.637	0.882	0.802

4. Conclusion

Reducing the concentration of Cr in contaminated soils can be performed by adding soil amendments. These amendments reduce Cr mobility by promoting the formation of insoluble precipitates or by enhancing the soil's capacity to bind Cr to the soil. The latter can be achieved directly through the addition of adsorbent materials or indirectly by adjusting the soil's pH–EC combination to provoke Cr absorption onto the soil. In this preliminary study, three factors were retained (pH, EC and DOC) in combination with four soil amendments (UNT, CPM, DL and DLX CPM). The present study confirmed that organic matter (CPM) is one of the best soil amendments, as it reduced the concentrations of Cr in the BL more than the other amendments. The interactions of the soil factors in the form of response surfaces were analyzed and compared to existing published results. This methodology could be effectively used to study the importance of individual, cumulative and interactive effects of the selected soil inputs in Cr concentration in bean leaves. The predictive ANN models used to evaluate

the Cr concentration corresponded well with the experimental results reported in the literature. The ANN models developed here showed that a plausible innovative low-cost strategy for in situ remediation of Cr contaminated soils using organic and inorganic amendments, modified vegetation, or a combination of the two approaches. It should be noted that the ANN approach proposed here does not take into account all the possible factors that may influence soil toxicity. The primary aim was to illustrate the potential of the ANN method in its ability for rapid and accurate prediction of soil toxicity. The ANN model can be extended by including additional factors and their combinations to capture complex Cr behavior.

Overall, the ANN modeling outperforms MLR modeling. The effort required to calibrate the ANN and MLR models were similar, and both require about the same amount of time and processing resources. Despite their good performance, ANNs suffer from a number of limitations. First, they are not able to explain the physical relationships between the input-output data. Secondly, there are no general guidelines for the design of ANN architecture for a given problem. There is a need for more research to develop such guidelines to enable the extensive application of ANNs for non-specialists. Nevertheless, ANNs have a number of significant benefits that make them a powerful, rapid and practical tool for solving many soil engineering problems. The implementation of ANN modeling for the robust and reliable prediction of metal concentration in plants using additional soil data such as soil particle size and moisture levels in a practical sensor system appears very promising. Future significant work is required to achieve this goal. The results of ANN testing (prediction) allow for the investigation of the soil factors' interactions. This methodology could therefore be effectively used to study the importance of individual, cumulative and interactive effects of the soil parameters. Moreover, the ANN method can be used in conjunction with mechanistic models at the research level as a method to determine the most important parameters in a design that could then be used to formulate a mechanistic model and to determine where future research efforts should be directed.

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Chapitre 3.2.

Identification inverse des traitements optimaux pour réduire la phytotoxicité du cuivre dans des sols contaminés phytorémédiés basée sur une modélisation par réseaux de neurones

3.2.1. Résumé de la publication

Dans notre travail appliqué sur le sol naturel, on a essayé d'évaluer la capacité de la phytostabilisation et de la phytoextraction assistée par des amendements organiques et minéraux à remédier les sols contaminés en éléments traces afin de diminuer l'exposition des êtres vivants du sol et notamment les plantes aux éléments traces. Afin d'optimiser le type et la quantité d'amendements utilisés pour remédier les sols contaminés dans le cadre de la phytostabilisation assistée, il est nécessaire de développer des outils de prédiction rapides et précis.

Dans cette étude, nous développons et testons un modèle neuronal artificiel inverse qui a pour objectif la prédiction du traitement du sol optimal pour réduire la toxicité du cuivre à partir d'entrées sélectionnées.

Pour réaliser notre objectif 16 échantillons de sol (quatre répétitions) ont été collectés à partir de 16 parcelles (1x3 m) amendées avec quatre types d'amendement: 4 fois le sol non traité (UNT), 4 fois le sol amendé avec 0,2% de calcaire dolomitique (DL), 4 fois le sol amendé avec 5% de compost du fumier de volaille et d'écorce de pin (CPM), et 4 fois le sol amendé avec un mélange de 0,2% DL avec 5% de CPM (DLX CPM). La toxicité est évaluée par une concentration cible donnée de Cu dans les feuilles de haricots nains (BL) cultivés en laboratoire, en pot, sur ces 16 échantillons de sol contaminés phytorémédiés. Après la récolte des haricots nains, l'humidité du sol a été maintenue à 80% pendant 15 jours. Après 15 jours la solution du sol (SPW) a été extraite par trois échantillonneurs Rhizon pour recueillir (30 ml) d'eau interstitielle du sol de chaque pot. La concentration en carbone organique dissous (COD) a été déterminée dans la solution du sol par un analyseur Shimadzu TOC 5000A. Le pH du sol et de la conductivité électrique (CE° ont été déterminés de la même solution du sol par pH-mètre et conductimètre.

Les entrées utilisées dans notre modèle sont le pH du sol, CE, DOC et une valeur cible donnée de toxicité par le cuivre alors que la sortie est le meilleur traitement pour réduire le niveau de toxicité donné.

Nos résultats ont montré que l'augmentation du pH dans les sols (UNT, CPM, DLX CPM) a conduit à une plus faible concentration du Cu dans les feuilles de haricots nains (BL) au même niveau de DOC tandis que l'effet inverse peut être observé dans le sol traité avec l'amendement DL.

Nous avons aussi trouvé aussi que le pH et le niveau de COD dans le sol non traité ont un effet significatif sur la concentration de Cu dans les feuilles de haricot par rapport aux sols traités. Nos

résultats ont également montré que l'ajout de CPM, DL et le mélange de ces deux amendements (DLXCPM) a diminué l'effet du pH du sol sur les concentrations de Cu dans les haricots quelle que soit la quantité de COD dans la solution du sol.

Notre travail a enfin montré que la méthode proposée peut réussir à identifier le meilleur traitement de sol à partir des propriétés du sol (entrées). L'amendement CPM a la capacité de réduire la toxicité du Cu quels que soient les valeurs de pH, EC et DOC dans le sol, alors que les amendements DL et DLX CPM ne sont pas en mesure de réduire la toxicité cible du Cu en-dessous de 200 $mg.kg^{-1}$ quelles que soient les valeurs de pH, EC et DOC. Pour réaliser notre travail, nous avons été confrontés à deux défis pour la prévision optimale du traitement à l'aide des réseaux de neurones, ces défis étant : la non-unicité de la solution du problème inverse et les inexactitudes dans la mesure des propriétés du sol (entrées). Notre modèle a démontré que le modèle de réseau neuronal de prédiction proposé peut surmonter ces deux défis. Il a été également démontré dans l'étude préliminaire que la méthode inverse en réseau de neurones peut potentiellement être appliquée avec un niveau élevé de succès dans l'étude des sols contaminés. Avant son application à grande échelle, une validation supplémentaire est cependant nécessaire en effectuant plusieurs expériences, y compris avec d'autres facteurs et leurs combinaisons, pour prédire le comportement complexe du sol.

3.2.2. Article 6 (*en revision dans Geochemical exploration journal*)

Application of an inverse neural network model for the identification of optimal amendment to reduce Copper toxicity in phytoremediated contaminated soils

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Abstract: Artificial neural network ANN prediction approaches applied to the modeling of soil behavior are often solved in the forward direction, by measuring the response of the soil (outputs) to a given set of soil inputs. Conversely, one may be interested in the assessment of a given set of soil inputs that leads to given (target) soil outputs. This is the inverse of the former problem. In this study, we develop and test an inverse artificial neural network model for the prediction of the optimal soil treatment to reduce copper (Cu) toxicity assessed by a given target concentration of Cu in dwarf bean leaves (BL) from selected soil inputs. In this preliminary study the inputs are the soil pH, EC, DOC and a given target toxicity value of Cu, whereas the output is the best treatment to reduce the given toxicity level. It is shown that the proposed method can successfully identify the best soil treatment from the soil properties (inputs). Two important challenges for optimal treatment prediction using neural networks are the non-uniqueness of the solution of the inverse problem and the inaccuracies in the measurement of the soil properties (inputs). It is shown that the neural network prediction model proposed can overcome both these challenges. It is also shown that the proposed inverse neural network method can potentially be applied with a high level of success to the phytoremediation of contaminated soils. Before large-scale application, further validation is needed by performing several experiments and investigations including additional factors and their combinations to capture the complex soil behavior.

Keywords: Soil contamination, Copper, Amendment optimization, Artificial neural networks (ANN), Inverse modeling

1. Introduction

Soils contaminated with trace elements have serious consequences for terrestrial ecosystems, agricultural production and human health (Adriano, 2001). Trace element contamination is considered as a negative effect of industrial activities which must be monitored, assessed and managed (Alloway, 1995). For instance several authors have reported that soil contamination is accompanied by a loss of biodiversity, land cover and finally a lack of nutrients and water (Freitas et al., 2004; Mench and Baize, 2004; Zvereva and Kozlov, 2007). The exposure of plants to contaminants causes the same consequences as environmental stress and results in a lower biomass and lower vegetation (Zvereva and Kozlov, 2004). According to the international organization for standardization, the bioavailability of soil contaminants is defined as the fraction of available contaminant in the soil acquired by a target-organism through physiological processes (Harmsen, 2007). Consequently, the characterization and prediction of metal phytoavailability in soils is a crucial step for assessing the efficiency of soil remediation strategies such as the addition of soil amendments including organic matter (compost, farm manure and biosolids), lime or other alkaline materials

(Oste et al., 2001; Lombi et al., 2003; Bolan et al., 2003; Brown et al. 2003; McBride, 1994; Puschenreiter et al., 2005; Ma et al., 2006) which have the capacity to adsorb, complex or (co)precipitate trace elements in the soil.

Various treatments can be suggested or tested experimentally with a view to reducing the toxicity of a specific contaminated soil to plants by observing the growth or death of the plants. It is therefore of immense practical importance to be able to determine the optimal soil treatment with an amendment to a specific soil in order to reduce the soil toxicity which can be controlled and measured by metal concentrations in vegetation. It is usual to try to predict the effectiveness of these treatments, e.g. by how much the metal concentration will be reduced in plant leaves. One can specify a maximum allowable limit value (target) of metal concentration in a specific soil and seek to identify the corresponding optimal treatment to reduce the toxicity below the specified target. Examples of the areas where such predictive capability is of great value are the monitoring and management of industrial sites. Thus it is necessary to develop rapid and accurate prediction tools to control and analyze contamination sites and to manage soil use. This requires an extensive data bank of soil input-output data. However, measuring these parameters is time-consuming, difficult and expensive. In such cases, there is no clear standard rule for selection of the optimal soil treatment and one needs to determine the response of the soil. This is the so called inverse problem identification which must be solved to answer the following question: what are the controlled inputs (e.g. amendments) that have resulted in this given output (metal concentrations in the plant leaves)?

The solution of the inverse problem has several practical applications in soil analysis, but has not been extensively studied so far, presumably due to the difficulties associated with the resolution of the nonlinear inverse problem. Over the last few years ANNs have been widely used in the field of soil science for the prediction of soil hydraulic properties (Schaap et al., 1998; Minasny et al., 2004), the generation of digital soil maps (McBratney et al., 2003; Behrens et al., 2005) and the modeling of the behavior of trace metals (Buszewski and Kowalkowski, 2006; Anagu et al., 2009; Gandhimathi and Meenambal, 2012). In this case, the ANN is trained to find these relations using an iterative calibration process. The ANN approach is beneficial compared to traditional regression methods if the input-output relationship is complex or unknown (Sarmadian and Taghizadeh Mehrjardi, 2008; Schaap and Leij, 1998; Hambli et al., 2006; Hambli, 2009; Hattab et al., 2013). Moreover, ANN can be used as an inverse modeling approach. ANN modeling has been previously applied for solving inverse problems in other engineering fields (Jenkins, 1997; Rafiq et al., 2001; Hambli et al., 2006), but has not been previously used in conjunction with soil analysis. Inverse ANNs have several advantages compared to other inverse identification techniques. First, ANNs are very general. It is proven that ANNs can accurately represent any sufficiently smooth nonlinear mapping (Jenkins, 1997; Rafiq et al., 2001). Second, the accuracy of the solution is independent of the number of inputs (Jenkins, 1997; Rafiq et al., 2001). This is an important point, because accurate prediction of the optimal soil treatment may require a large number of soil inputs. Third, ANNs are particularly useful in cases where solving the forward problem model is time-consuming (Hambli et al., 2006).

In this study, we have developed and tested an inverse artificial neural network (ANN) model for the prediction of optimal soil treatment to reduce toxicity assessed by a given target concentration of Cu in dwarf bean leaves (BL) from a given set of soil properties (inputs).

In order to prepare the training data for the inverse ANN, 16 (4x4) soil samples were collected from different soil profiles from a Cu sulfate and Chromated Copper Arsenate (CCA) contaminated site located in south-western France. The measured soil variables were soil pH, soil electrical conductivity (EC), dissolved organic carbon (DOC) and the concentration of Cu in BL grown in the laboratory on these contaminated soils treated with inorganic and organic amendments, with 4 replications for each measurement (4*4 measurements). The inverse ANN model was then

developed and trained to predict the best soil treatment. The inputs were the soil pH, EC, DOC, and a given target toxicity value of Cu, whereas the output is the best treatment to reduce the given toxicity level.

Two important aspects in the estimation of the optimal soil treatment from the measured soil inputs is the non-uniqueness of the solution of the inverse problem and the inaccuracies that may exist in the measurement of the soil inputs. The non-uniqueness of inverse solutions is a challenge for any inverse problem algorithm, because several solutions exist for the same inverse problem. The convergence of the solution may therefore be compromised. The second challenge is the inaccuracies that may exist in the actual measurements of soil inputs. The inverse ANN algorithm should be robust enough to be able to provide reasonable predictions of optimal soil treatment even when the soil input measurements are not perfectly accurate. Both challenges are addressed for the proposed inverse ANN algorithm. Results show that the inverse ANN model leads to a rapid and accurate prediction of the optimal soil treatment.

2. Material and Methods

From a practical point of view, the following three steps are required for the development of the inverse ANN model:

(i)Performing suitable experiments to measure the effects of selected soil inputs (properties, inorganic and organic amendments) on the soil toxicity assessed by concentration of Cu in dwarf BL.

(ii)Forward training the neural network based on the results of step (i) (mapping inputs to outputs).

(iii)Inverse ANN prediction (Prediction of the inputs given a target set of outputs).

The present section of the paper is divided into three sub-sections. The first sub-section presents the soil experiments. The second sub-section describes the inverse ANN approach and the third sub-section deals with the inverse ANN prediction considering the non-unique solutions of the inverse problem.

2.1. Soil sampling and preparation

16 soil samples (four replicates) were collected from 16 plots (1x3 m) from the BIOGECO phytostabilization platform installed on a former wood preservation site located in south-western France, Gironde County (44°43'N; 0°30'W). This site has been contaminated with high concentrations of Cu. The history of the site and its characteristics are detailed in (Mench and Bes, 2009; Bes et al., 2010). Long-term aided phytostabilization experiments are established at the site (Bes *et al*. 2010). After that four different amendments were applied on the site and carefully mixed in the topsoil (0-0.30 m) with a stainless steel spade with four replicates: untreated soil (UNT), 0.2% of dolomite limestone (DL), 5% of compost of poultry manure and pine bark (CPM), and a mixture of 0.2% DL along with 5% CPM (DLX CPM). Sixteen soil samples were collected from the topsoil of the platform, to a depth of 0.25 m. One kilo of each soil was placed in a pot after sieving (2 mm). Four seeds of dwarf beans (*Phaseolus vulgaris*) were sown in all pots and cultivated for 18 days in controlled conditions (16 h light/8 h darkness regime). The soil moisture was maintained at around 50% of the field water capacity with additions of distilled water after weighing. Then the soil moisture was raised to 80% (11-13 % of air-dried soil mass) at the beginning of the germination stage of the seeds. At the end of the growing period the plants were harvested, and then the dry weight of BL was determined after drying at 70C°.

The BL were weighed (35-150 mg) directly into Savillex Polytetrafluoroethylene PTFE 50mL vessels, 2 ml H_2O and 2mL supra-pure 14 M HNO_3 were added and the vessels were heated open at 65C for 2 hours. Then the caps were closed and the containers were left overnight at 65 C° (12-14 h). After that they were opened, 0.5mL of H_2O_2 (30%) was added to each sample and left at 75 C° open for 3 hours. Then 1.5+/-0.5ml of ***Fluorhydric Acid*** HF (48%) was added to each sample, caps were closed and left at 100 C° overnight. Containers were

opened and kept at 120 C° for 4-5 hours evaporating to dryness, taken off heat, 1mL HNO_3 + 5ml H_2O + 0.1 ml H_2O_2 were added to each, gently warmed up and after cooling down made up to 50 ml. Mineral composition in BL was determined by ICP-MS (Varian 810-MS).

2.2. Characterization of soil solution

After harvesting the dwarf beans, the soils was watered with distilled water, and daily maintained at 80% of field capacity (11-13% of air-dried soil mass) for 15 days. After 15 days three Rhizon soil-moisture samplers (SMS) from Rhizosphere Research Products (Wageningen, Holland) were inserted for 24 h with a 45° angle into each potted soil (3x16 soils) to collect (30 mL) soil pore water from each pot. Then dissolved organic carbon (DOC) was analyzed in the soil solution by a Shimadzu© TOC 5000A analyzer. Soil pH and EC were determined in the same soil solution by pH meter and the electrical conductivity meter (EC).

2.3. Neural network method

The ANN architecture is composed of an input layer, a certain number of hidden layers and an output layer in forward connections. Each neuron in the input layer represents a single input parameter. These values are directly transmitted to the subsequent neurons of the hidden layers. The neurons of the last layer represent the ANN outputs (Fig. 1).

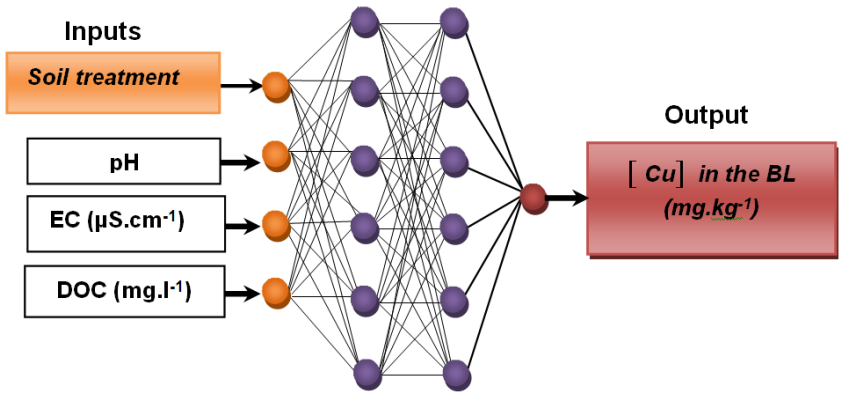


Figure 1. Direct neural network architecture for standard forward modeling composed of 4 inputs, two hidden layers and one output layer.

The output y_i^m of neuron i in a layer m is calculated by (Jenkins, 1997; Rafiq et al., 2001; Hambli et al., 2006):

$$y_i^m = f\left(v_i^m\right) \quad (1)$$

$$v_i^m = \sum_{j=1}^{L} w_{ji}^{m-1}\, y_j^{m-1} + b_i^m \quad (2)$$

Where f is the activation function, L is the number of connections to the previous layer, w_{ji}^{m-1} corresponds to the weights of each connection and b^m is the bias, which represents the constant part in the activation function.

From among activation functions the sigmoid (logistic) function is the most usually employed in ANN applications. It is given by (Jenkins, 1997; Rafiq et al., 2001; Hambli et al., 2006):

$$f\left(v_i^m\right) = \frac{1}{1+\exp\left(-\theta v_i^m\right)} \quad (3)$$

Where θ is a parameter defining the slope of the function ($\theta = 0.9$).

2.2.1 Training algorithm

The training process in the ANNs involves presenting a set of examples (input patterns) with known outputs (target output) (Jenkins, 1997; Rafiq et al., 2001; Hambli et al., 2006; Hambli, 2009). The system adjusts the weights w_{ji}^{m-1} of the internal

connections to minimize errors between the network output and target output. There are several algorithms in an ANN and the one which was used here is the back-propagation (BP) training algorithm of type Levenberg-Marquardt. BP algorithm is an iterative gradient algorithm designed to compute the connection weights minimizing the total mean-square error between the actual output of the multi-layer network and the desired output. The knowledge is represented and stored by the strength (weights) of the connections between the neurones.

In the present work, an in-house ANN program called Neuromod written in Fortran (Hambli et al., 2006; Hambli, 2009) has been applied. The basic ANN configuration employed in this study is selected to have one hidden layer with four neurons with learning rate factor $\eta = 0.1$ and momentum coefficient $\alpha = 0.1$. Tests performed for more than one hidden layer and different η and α parameters showed no significant improvement in the obtained results. The learning rate coefficient η and the momentum term α are two user-defined BP algorithm training parameters that affect the learning procedure of ANN. The training is sensitive to the choice of these net parameters. The learning rate coefficient employed during the adjustment of weights (w_{ji}^{m-1}) is used to speed-up or slow-down the learning process. A bigger learning coefficient increases the weight changes, hence large steps are taken towards the global minimum of error level, while smaller learning coefficients increase the number of steps taken to reach the desired error level.

To prepare the training data for the ANN, different measurements were performed on the four different soils subjected to four different treatments (UNT, DL, CPM and DLX CPM). The input factors and their minimum/maximum levels are given in Table 1.

40 measures were used for training, 16 samples for testing and 8 samples for validation. The testing data were not used for training. The testing data provided cross validation during the ANN training for verification of network prediction accuracy.

Table 1. Selected inputs and output for ANN training.

Inputs	**Level**	**Min value**	**Max value**
Soil treatment	4	#1	#4
pH	4	6.97	7.55
EC (µS.cm^{-1})	4	111	208
DOC (mg.l^{-1})	4	26.75	42.724
Output			
***Cu* concentration in the bean leaves (mg.kg^{-1})**			

The validation data are used to measure the ANN performance of its predicting capability after complete training. In order to avoid data saturation, the inputs and the output variables were normalized between 0 and 1 using:

$$x_i^{norm} = \frac{x_i - x_i^{\min}}{x_i^{\max} - x_i^{\min}} \qquad (4)$$

Where x_i , $x_i^{\min}$, $x_i^{\max}$ and x_i^{norm} denote respectively, the real input (output) variables value *i*, the minimum input (output) variable, the maximum input (output) variable and the normalized value *i*.

The real (de-normalized) value of the ANN is computed using:

$$y_t = y_{\min} + y_{norm}\left(y_{\max} - y_{\min}\right) \qquad (5)$$

Where y_t , $y_{\min}$, $y_{\max}$ and y_{norm} are the real-valued output variable, the minimum and maximum values of the real-valued output and the normalized output value from the neural- ANN model respectively.

In the current preliminary study, we selected a limited number of independent soil parameters: soil pH, CE, soil solution's DOC and a given target toxicity value of Cu concentration. Whereas the output is the optimal amendment to reduce the given toxicity level of Cu.

It should be noted that the proposed ANN approach does not take into account all possible factors which may influence the soil toxicity. The prediction based on the ANN depends on the inputs pattern. Therefore, adding more inputs would lead to more accurate and reliable results.

The primary aim here was to illustrate the potential of the inverse neural network method to predict optimal soil amendment to reduce its toxicity rather than performing investigation related to the effect of a larger soil inputs.

2.2.2. Soil factors selection

The correlations among soil variables may significantly affect the predictions of soil outputs. Therefore, attention must be taken concerning the selection of soil parameters. Although the parameter correlations are observed and may be strong in some cases, existing soil analysis methods typically adopt the assumption of independent parameters (van der Zee and van Riemsdijk, 1987; Andersen and Christensen, 1988; Streck and Richter, 1997; Römkens and Salomons, 1998; Tiktak et al., 1998; Schaap and Lei, 1998; Elzinga et al., 1999; Annadurai and Lee, 2007; Sarmadian and Taghizadeh Mehrjardi, 2008; Schaap et al., 1998; Minasny et al., 2004; McBratney et al., 2003; Behrens et al., 2005; Buszewski and Kowalkowski, 2006; Anagu et al., 2009 ; Gandhimathi and Meenambal, 2012).

In this preliminary study, the soil inputs for the ANN model were limited to the case of three most influential independent factors (predictor variables) on the mobility and availability of metals in the soil:

-Soil pH: Kabata-Pendias et al., (2000) and Jackson et al., (2000) reported that the addition of amendments to soil increased the soil pH compared to the untreated soil, the thing that led to the reduction the mobility and the availability of metals to plants.

-DOC: Hsu et al., (2000) noted that the addition of amendment to the soil increased the contain of the soil of the DOC compared to untreated one, which lead to increasing the mass vegetal of plant, reducing the bio availability of metals by formed legends with the dissolved organic matter.

-EC: Du Laing et al., (2008, 2009) and Hatje et al., (2003) found that the increase in conductivity (salinity) has led to increase the mobility of some metals such as cadmium and zinc, thereby increasing the availability and the bioavailability of these metals to the plant.

Correlative models like ANN ones are applied to approximate various complex engineering problems. Model development starts with the determination of parameters which are most influential on model results. One may perform sensitivity analyses to determine: (1) which parameters require additional future research; (2) which parameters are insignificant and can be eliminated from the final model; (3) which inputs contribute most to output variability; (4) which parameters are most highly correlated with the output and (5) once the model is in production use, what consequence results from changing a given input parameter (Hamby, 1993). Model parameters exerting the most influence on model results are identified through a sensitivity analysis. In our case, the correlations among soil variables may significantly affect the predictions. In such a case, decomposition methods can be used to reduce the prediction sensitivity (Jacques et al., 2006).

2.3. Inverse neural network for inverse prediction

In general, standard ‘forward mapping’ using ANN involves a nonlinear mapping from the space of the soil inputs to the soil outputs (toxicity). The inverse of the forward mapping goes backward in time. Given a certain soil target response, the inverse prediction based on ‘backward mapping’ determines the set of soil inputs that has resulted in the given soil outputs,

thereby mapping the space of soil outputs to the space of soil inputs (Fig. 2).

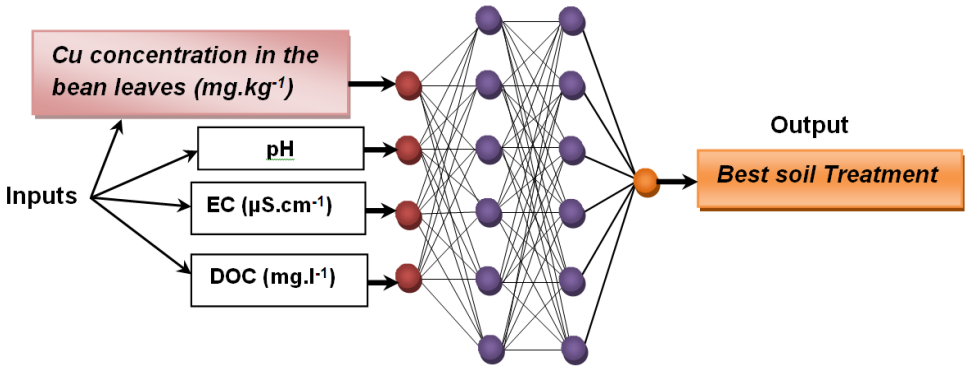


Figure 2. Inverse ANN model for backward modeling composed of 4 inputs, two hidden layers and one output layer.

It is mathematically proven that a feed forward ANN with at least one hidden layer, *n* hidden neurons, and sigmoid activation functions can approximate any continuous function with an integrated squared error regardless of the dimension of the input space (Barron, 1993).

2.4.1. Non-uniqueness and robustness of the inverse ANN model

The non-uniqueness of the solution is an important challenge for all inverse identification methods because several solutions exist for the same inverse problem. In the current work, we assign a search interval (ΔY_t) to the given target soil output (toxicity) (Y_t) and the inverse ANN was designed to automatically identify via iterative loops all the corresponding inputs which generate outputs located within the interval ΔY_t.

A second challenge is that the measurements of soil inputs and corresponding outputs are not noise-free. It is therefore important that the inverse ANN model be robust enough to handle the noise that will exist in actual soil measurements. In the current work, to ensure the robustness of the inverse ANN, the trained ANN was tested using noisy output data in the form:

$$Y_t^n = Y_t + f\left(0,\ Y_t / \lambda\right) \qquad (6)$$

where Y_t^n denote respectively the noisy output , $f\left(0,\ Y_t / \lambda\right)$ is the standard Gaussian distribution function (mean value: $\mu = 0$ and standard deviation: $\sigma = Y_t / \lambda$). The signal to noise ratio is represented by Y_t / λ.

3. Results and Discussion

The inverse ANN was trained with 5.10^5 epochs. The training performance was assessed by the root mean square error (RMSE). At the end of the training phase, the RMSE convergence value was $1.E^{-5}$. The inverse ANN was then tested without considering the noisy dataset:

(i) First, the direct ANN was applied to 300 testing combinations of soil inputs to predict the optimal soil amendments (considered as the reference values by the direct ANN prediction).

(ii) Then, the inverse ANN was applied to predict the optimal soil amendment related to these 300 testing combinations.

(iii) The results generated by the inverse ANN in step (ii) were compared to the direct ANN results of step (i). Figure 3 shows the variation of the inverse predicted optimal amendments versus the reference ones. The integers 1, 2, 3 and 4 refer to UNT, CPM, DL and DLX CPM soil amendments respectively

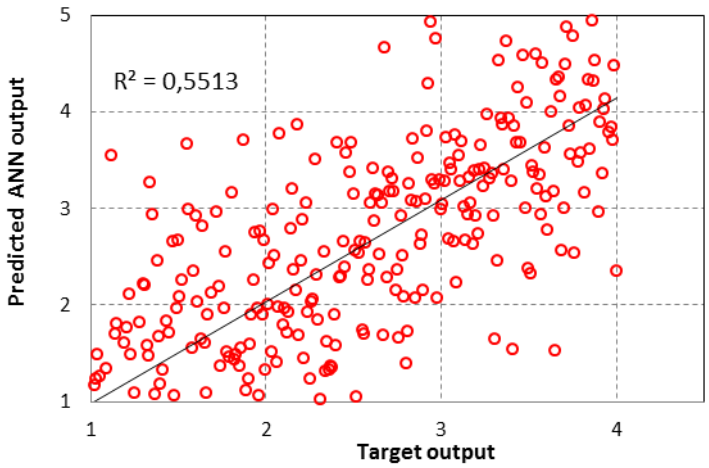


Figure 3. Inverse ANN prediction versus actual soil inputs obtained by an Inverse ANN trained using noise-free training data. Results were obtained for 300 testing combinations of soil inputs. Outputs 1, 2, 3 and 4 refer to UNT, CPM, DL and DLX CPM soil amendments respectively.

It was found that the ANN is sensitive to noise and gives inaccurate output predictions when the input dataset is slightly noisy. It can be noticed that the simulations generated solutions composed of mixed amendments as optimal treatment (non-integer values in the curve denote mixed amendments). For example 1.5 represents a 50% mix of 1 (UNT) and 2 (CPM). In previous studies, numerous amendments have been incorporated into soils polluted with metal (metalloids) to immobilize pollutants. These amendments include individual additions of amendments (Walker et al., 2004; Schwab et al., 2007; Derome, 2009; González-Alcaraz et al., 2011; Melamed et al., 2003; Brown et al., 2005; Ford, 2002; Trivedi and Axe, 2001; Hartley and Lepp, 2008; Gupta and Sharma., 2002; Garau et al., 2011; Brown et al., 2003; Torri and Lavado, 2008) and combinations of different amendments including organic and liming materials (Alvarenga et al.,2008; Farrell and Jones, 2010; Bes and Mench, 2008), iron oxides and lime (Warren and Alloway, 2003) and compost and iron oxide (Gadepalle et al., 2008).

In order to reduce the sensitivity of the inverse ANN to noise, the training algorithm was modified by introducing noise to the training and validation datasets (Eq. 6) with signal to noise ratios between 100 and 5 (Fig. 4). It was found that when ANNs are trained using noisy datasets, they are very robust and work well even when the input and output values are noisy. As depicted in Fig. 4, the accuracy of the inverse ANN prediction decreases when the signal to noise ratio (λ) decreases (lower R^2 value). However, the reduction in accuracy with the increase in noise is gradual and simply reflects the lower quality of the measurement data. This result indicates that the inverse ANN model was able to predict accurately the optimal soil treatment corresponding to a target value of Cu concentration in the BL.

Because of the noise (uncertainty) concerning the input measurements which can be characterized by a given set of scattered input data with a measured statistical distribution (mainly a mean and standard deviation), robust processing of scattered data methods is needed to predict reliable responses. Using the inverse ANN model developed, this can be performed based on a three-stage analysis:

(i) Perform simple input-output predictions on the scattered data set to obtain initial measures of output variations. This evaluation does not account for statistical noise.

(ii) Generate stochastic data based on the mean value and the standard deviation and perform a stochastic prediction.

(iii) Estimate the sensitivity of the stochastic results of (ii) in relation to the results of (i) and assess the impact of the input sensitivities on the response.

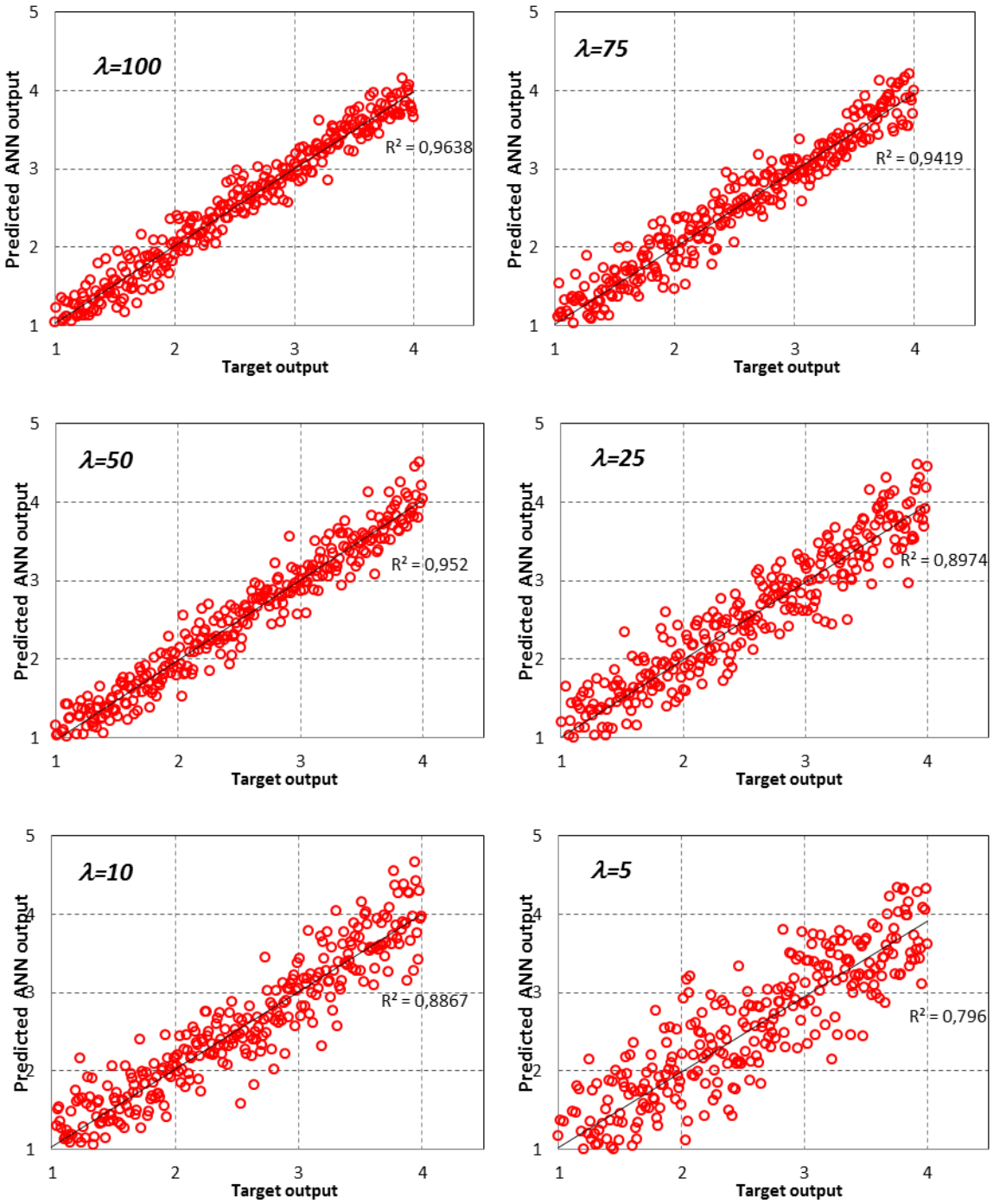


Figure 4. Inverse NN prediction versus actual soil inputs obtained by an ANN trained using noisy training data with decreasing levels of noise. Results were obtained for 300 testing combinations of soil inputs. Outputs 1, 2, 3 and 4 refer to UNT, CPM, DL and DLX CPM soil amendments respectively.

In this way, a confidence interval can be estimated related to the data noise. Note that enhanced data smoothing procedures can be applied in order to identify general trends by removing the background noise of random variability often present in raw data and to increase the prediction accuracy (Velleman, 1980; Kafadar, 1994). There are a number of smoothing algorithms including histograms, nearest-neighbor, kernel and regression smooths. The selection of a suitable procedure depends mainly on its robustness to treat the scatter, its speed and its interpretation.

In order to test the capability of the trained inverse ANN to predict the optimal treatment, the model was run for different combinations of soil

inputs. The input parameters were soil pH, soil EC and DOC input factor, four values in the interval of its min and max value were applied here to capture the non linear soil response (Table 2).

256 ($4\times4\times4\times4$) full factorial combinations of the inputs were computed with the trained inverse ANN with a total computation time of about 2 seconds.

Figure 5 shows the predicted optimal treatment for soil corresponding to a target value of Cu concentration in BL of 150 mg.kg^{1}. The non-uniqueness of the solution related to the effects of soil pH and DOC can be observed corresponding to the target Cu concentration indicated by the vertical dashed line ($\lambda = 50$).

Table 2. Selected four inputs for the inverse ANN prediction. Full factorial combinations generate 256 ($4\times4\times4\times4$) data set.

Inputs	min	max	Level 1	Level 2	Level 3	Level 4
Target Cu concentration (mg.kg^{-1})	10	500	100	200	300	400
pH	6.97	7.55	7.0	7.1	7.2	7.3
EC (µS.cm^{-1})	111	208	120	140	160	180
DOC (mg.l^{-1})	26.75	42.724	28	32	35	40

The results show the dependence between the soil properties (selected inputs in the current case) and the optimal treatment of the soil to reduce the Cu plant toxicity (case of 150 mg.kg^{-1}). One can see in the (UNT, CPM, DLX CPM) soils that increasing the soil pH leads to a lower concentration of Cu in the bean leaves at the same level of (DOC), while the opposite effect can be observed in the soil treated with (DL).

It can be seen that the acidity and the level of DOC in the untreated soil significantly affect the Cu concentration in the bean leaves compared to the treated soils. Predicted results show that the addition of CPM, DL and DLX CPM amendments to the soil decrease the effect of the soil pH on the concentrations of Cu in the bean leaves whatever the quantity of DOC in the soil solution.

The plots on Fig. 5 show that the best amendment for decreasing the concentration of Cu in the bean leaves under the effect of the selected input factors (pH, DOC, and EC) and for a given target value of Cu in the BL (150 mg.kg^{-1}) is the CPM which generated a significant reduction in Cu concentrations in the BL compared to the other treatments. CPM reduced the concentrations of Cu in the BL to less than 200 mg.kg^{-1}, followed by the soil treated with DL (concentrations of about 270 mg.kg^{-1}), then the soil treated with the DLX CPM (concentrations of almost 285 mg.kg^{-1}), while the Cu concentrations in the UNT soil reached a maximum value of about 300 mg.kg^{-1} for a soil pH of 7 and less than 150 mg.kg^{-1} for a soil pH of 7.3. This result suggests that soil pH must be assessed accurately for the optimal monitoring of soil Cu toxicity.

Our predicted results agree with the results of Harter and Naidu (1995) who explained this observation by the capacity of CPM in the soil to retain soil metals.

The bioavailability of Cu in the soil is considered as the part of the metal which is found free in the solution, i.e. not complexed with organic species nor adsorbed to the soil solid fractions (Zhang et al., 2001). The binding of the metal to the organic fraction depends on the total number of both strong and weak binding sites, which is related to the number of functional groups. The total acidity e.g. the number of carboxylic acid and phenol hydroxyl groups is often used as a measurement of binding capacity (Gerke, 1994). Sauvé et al. (2000) reported that the availability of soil metals to the plants depends on two factors: (i) the presence of several sorbents in the soil such as organic matter, and (ii) the physico–chemical parameters such as pH and ionic strength. Kabata-Pendias and Pendias (1992), Lexmond (1980), McBride (1989), Tyler

and Olsson (2001) reported that the bioavailability and toxicity of Cu in the soil is increased in acidic soil relative to calcareous soil. This increase is due to the increase in the concentration of Cu^{+2} (free Copper) in the soil solution which generally presents the available form of Cu to the living organisms in the soil (plants, microorganisms). The increase in the soil pH leads to an increase in the binding of Cu to the soil constituents (McLaren and Crawford, 1973) and therefore decreases the mobility and availability of soil Cu. Brown et al. (2003) found that the addition of organic amendments to the soil such as compost, farmyard manure and biosolid compost reduced the availability of trace metals in soils due to the high content of organic matter, P and Fe. Balasoiu et al. (2001) reported that the organic matter of soils can bind significant amounts of Cu by forming an OM-bound Cu fraction which represents about 96% of the total Cu in CCA-contaminated soil.

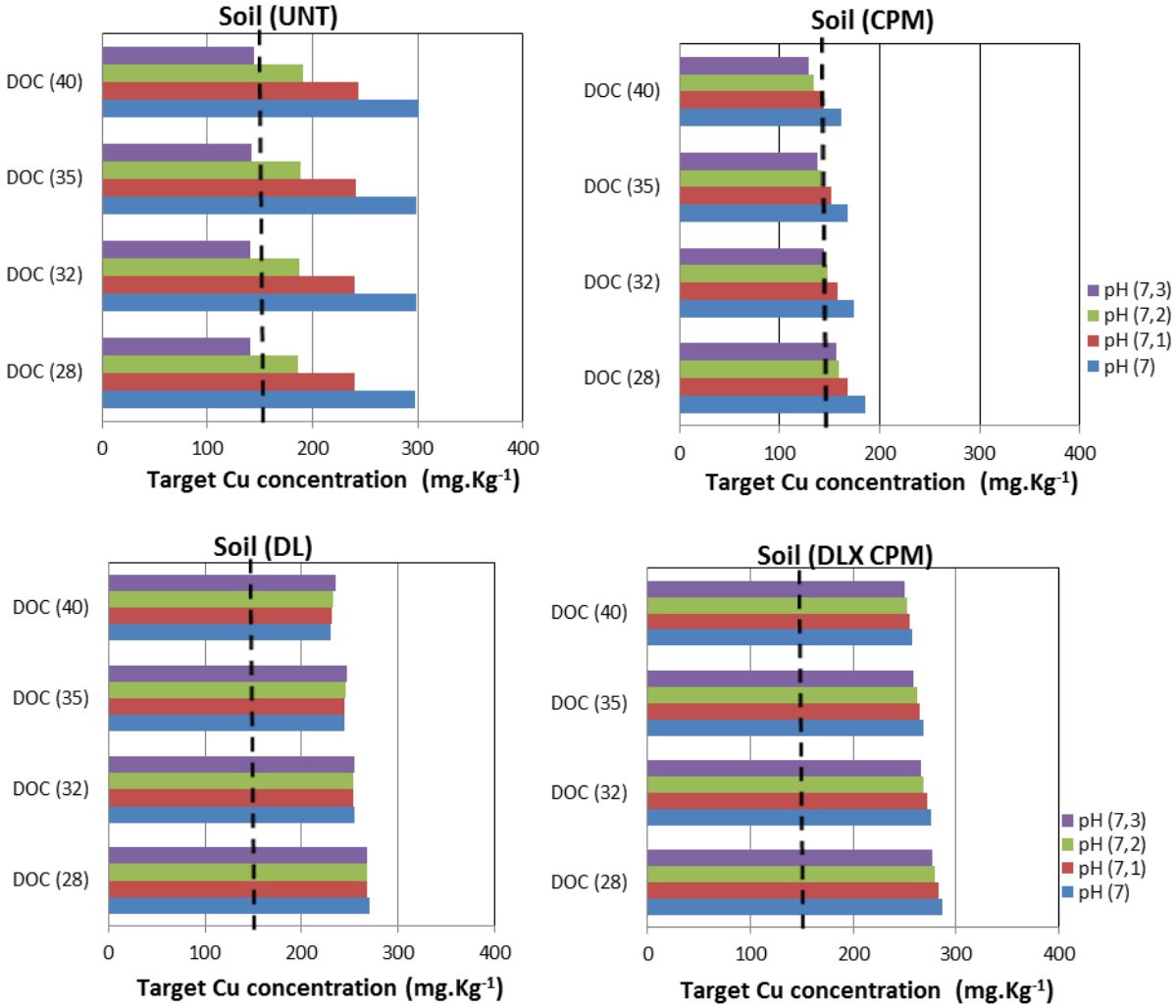


Figure 5. Predicted optimal treatment for soil corresponding to a target value of Cu concentration in BL of 150 $mg.kg^{-1}$. Non-uniqueness of the solution related to the effects of soil pH and DOC can be observed corresponding to the target Cu concentration indicated by the vertical dashed line ($\lambda = 50$). For illustration, the EC was fixed at $\mu S.cm^{-1}$.

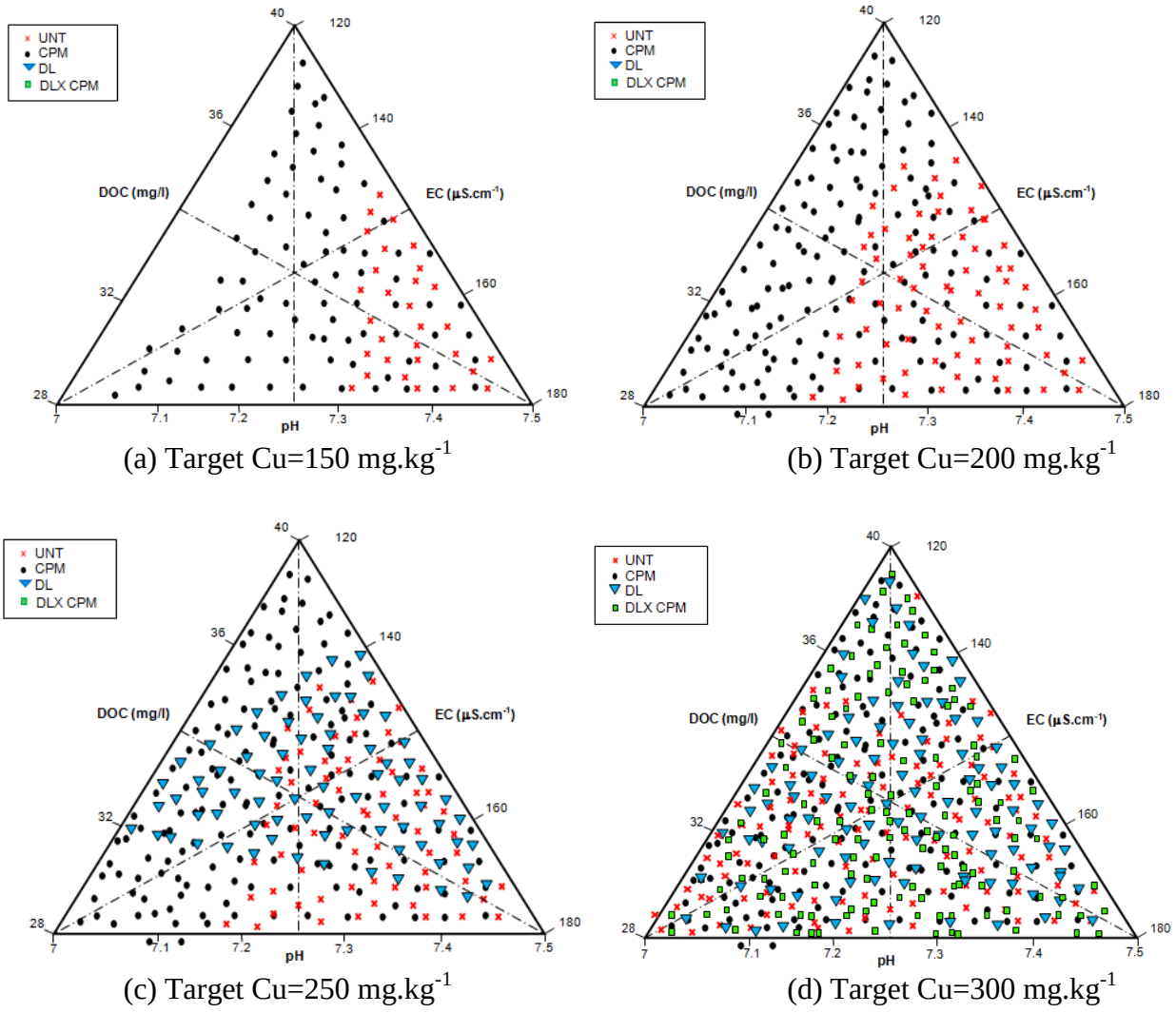
Figure 6 shows the predicted optimal treatments for the soil in the form of a ternary (triangular) plot corresponding to different target values of Cu concentration in BL and soil pH, EC and DOC interactions ($\lambda = 50$). The ternary diagram is a graph that shows the response of three combined variables (pH, EC and DOC) as a position in an equilateral triangle. Such diagrams can be used for graphical classification and interpretation of soil responses. The advantage of using a ternary plot for depicting compositions is that the three variables can be conveniently plotted

in a two-dimensional graph. Every point on a ternary plot represents a different composition of the three components. It can be used for soil treatment classification schemes.

The ternary diagram indicates that the UNT soil is the best treatment to reduce toxicity below the target Cu value of 150 $mg.kg^{-1}$ corresponding to a soil pH greater than 7.3 combined with soil EC greater than 140 $\mu S.cm^{-1}$ (red crosses) (Fig. 6-a) independent of the DOC value. For lower values of soil EC and of pH (7.1), only the CPM treatment is able to reduce the toxicity (black circles) (note the empty region in Fig. 6-a).

When the target Cu value is increased to 200 $mg.kg^{-1}$ (Fig. 6- b), the inverse ANN predicted the same results but with a larger region in the plot, indicating that the UNT is the most suitable to reduce toxicity for a soil pH higher than 7.2. The CPM treatment is able to reduce the toxicity (black circles) whatever the values of pH, EC and DOC. Note that DL and DLX CPM are unable to reduce the target Cu toxicity below 200 $mg.kg^{-1}$ whatever the values of pH, EC and DOC. For a target Cu value of 250 $mg.kg^{-1}$ (Fig. 6-c), the predicted results reveal that the UNT soil domain remains unchanged (saturation), indicating that UNT soil is unable to reduce the toxicity outside the range (pH<7.2 and EC<140 $\mu S.cm^{-1}$). The CPM treatment is able to reduce the toxicity (black circles) whatever the values of pH, EC and DOC. It can also be seen that the DL treatment (blue triangles) is able to reduce the toxicity below a Cu value of 250 $mg.kg^{-1}$ in the range of (DOC<35 $mg.l^{-1}$ and EC>140 $\mu S.cm^{-1}$). Finally, for a larger target value of Cu (300 $mg.kg^{-1}$) (Fig. 6-d), all four treatments are able to sreduce the toxicity except in the case of UNT soil for EC<140 $\mu S.cm^{-1}$.

The ternary diagram shows that among the four treatments, CPM leads to the best results (covering the largest region in the plots). Nevertheless, the other treatments can be considered as optimal choices for particular cases depending on the combinations of the pH, EC and DOC values.



(a) Target Cu=150 mg.kg^{-1} (b) Target Cu=200 mg.kg^{-1}

(c) Target Cu=250 mg.kg^{-1} (d) Target Cu=300 mg.kg^{-1}

Figure 6. Ternary diagram to classify the predicted optimal treatments for soil corresponding to different target values of Cu concentration in BL: Effects of soil pH, EC and DOC interactions.

4. Conclusion

Several models have been developed to predict the relationship between soil input parameters and design performance of ANNs. Generally, two methods are used to develop prediction models: conventional mathematical methods and ANN. It has been reported that ANNs provided superior predictive performance when compared with conventional mathematical methods including multiple linear regression (MLR models) (van der Zee and van Riemsdijk, 1987; Andersen and Christensen, 1988; Streck and Richter, 1997; Römkens and Salomons, 1998; Tiktak et al., 1998; Schaap and Leij, 1998; Elzinga et al., 1999; Annadurai and Lee, 2007; Sarmadian and Taghizadeh Mehrjardi, 2008). In addition, when MLR methods are used, the relationships between soil inputs and design outputs have to be stated a priori in the regression models. In many situations in soil engineering, the input-output relationships are highly complex and are poorly understood. The lack of physical understanding and of a powerful general tool for mathematical modeling leads to either simplifying the problem or incorporating several assumptions into the mathematical models. Consequently, many mathematical models fail to simulate the complex behavior of most soil engineering problems. In contrast, ANNs are based on the data alone where such relationships do not need to be formulated beforehand (Schaap et al., 1998; Minasny et al., 2004; McBratney et al., 2003; Behrens et al., 2005; Buszewski and Kowalkowski, 2006; Anagu et al., 2009; Gandhimathi and

Meenambal, 2012). Moreover, ANNs can be used at research level as a method to determine the most important parameters in a design that could then be used to formulate a mechanistic model and to determine where future research efforts should be targetted. The results of ANN testing (prediction) allow for the investigation of the soil factors' interactions. This methodology could therefore be effectively used to study the importance of individual, cumulative and interactive effects of the selected soil inputs in the phytoremediation of contaminated soil measured by metal concentrations in vegetation. The effect of several soil treatments and their combinations can also be investigated. For example, the proposed inverse ANN model generated solutions composed of mixed amendments as the optimal treatment (Fig. 3) which is in agreement with previous studies where the authors applied individual combinations of different amendments (Alvarenga et al.,2008; Farrell and Jones, 2010; Warren and Alloway, 2003; Gadepalle et al., 2008; Bes and Mench, 2008). The present study showed that a plausible innovative low-cost strategy for the optimal remediation of contaminated soils in situ could be performed using such inverse ANN approaches.

The effects of noise on the performance of the ANN were also investigated. Noise was introduced to the training data points and the inverse ANN was trained using noisy inputs. It was observed that the proposed modification in the training algorithm makes the ANN very robust against noisy density measurements. It was shown that the ANN works reasonably well even when both non-uniqueness and noise are taken into account.

Despite their good performance in many situations, ANNs suffer from a number of limitations. First, they are not able to explain the physical relationships between the input-output data. Second, there are no general guidelines which can help in the design of the ANN architecture for a given problem.

The primary aim of the current study was to illustrate the potential of the inverse neural network method as an alternative to traditional mathematical models to predict the optimal soil amendment to reduce soil toxicity. The inverse problem (going from the target Cu level and soil properties to the best amendment for reducing soil toxicity) has not a unique solution. The inverse ANN developed was specially designed for the prediction of non-unique optimal soil treatments. The ANN method can potentially be applied with a high level of success in the phytoremediation of contaminated soils. Before its large-scale application, however, further investigations are needed by performing: (i) experimental validation based on several experiments including additional factors to capture complex soil behavior, (ii) a sensitivity analysis to check the reliability of the inverse ANN and (iii) enhanced data smoothing procedures in order to identify general trends by removing the background noise of random variability often present in raw data and to increase the prediction accuracy.

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Chapitre 3.3.

Effet de la variabilité des paramètres chimiques du sol sur la variabilité de la concentration du cuivre phytodisponible dans un sol contaminé phytorémédié à l'aide d'une modélisation hybride par réseaux de neurones et simulations de Monte Carlo

3.3.1. Résumé de la publication

Dans l'article précédent nous avons essayé de prédire les concentrations phytodisponibles du Cr en fonction de l'effet de differents paramètres du sol et des amendements sur un sol contaminé phytostabilisé.

L'objectif de cet article est d'etudier l'effet de la variation statistique des paramètres du sol comme le pH, DOC et CE généré par l'addition de différents amendements au sol dans le cadre de la phytostabilisation assistée sur la mobilité, la biodisponibilité et la concentration de cuivre dans feuilles de haricots nains cultivées en laboratoire sur les sols contaminés phytorémédiés.

Notre travail a porté sur le même sol naturel étudié précédemment. Pour réaliser notre objectif 16 échantillons de sol (quatre répétitions) ont été collectés à partir de 16 parcelles (1x3 m) amendées avec quatre types d' amendement: 4 fois le sol non traité (UNT), 4 fois le sol amendé avec 0,2% de calcaire dolomitique (DL), 4 fois le sol amendé avec 5% de compost du fumier de volaille et d'écorce de pin (CPM), et 4 fois le sol amendé avec un mélange de 0,2% DL avec 5% de CPM (DLX CPM). La toxicité est évaluée par une concentration cible donnée de Cu dans les feuilles de haricots nains (BL) cultivés en laboratoire, en pot, sur ces 16 échantillons. Après la récolte des haricots nains, l'humidité du sol a été maintenue à 80% de la capacité au champ pendant 15 jours. Après 15 jours la solution du sol à été extraite par trois échantillonneurs Rhizon pour recueillir (30 ml) d'eau interstitielle du sol de chaque pot. La concentration en carbone organique dissous (COD) a été analysée dans la solution du sol par un analyseur Shimadzu TOC 5000A. Le pH du sol et de la conductivité électrique (CE) ont été également déterminés dans la même solution de sol.

Notre méthode de travail est basée sur une technique de modélisation hybride utilisant un réseau de neurones artificiels (ANN) et des simulations de Monte Carlo (MCS). Alors que les analyses répétées requises par les MCS prennent beaucoup de temps, le RNA est utilisé pour prédire la concentration de cuivre dans feuilles de haricots nains en réponse aux combinaisons d'entrées du sol. Les données d'entrée pour l'ANN sont un ensemble de paramètres sélectionnés du sol générés aléatoirement selon une distribution gaussienne pour représenter les incertitudes des paramètres. La sortie est la concentration de cuivre dans les feuilles de haricots nains.

Les résultats ANN-MCS ont indiqué que l'incertitude du pH a généré la plus forte variabilité de la concentration du Cu mesuré dans l'haricot, suggérant que le pH joue un rôle principal dans la stabilisation du processus de phytoremédiation. Le deuxième facteur influent était la DOC. Nous avons constaté aussi que la CE était le facteur le moins influent par rapport au pH et DOC.

La simulation a indiqué que l'amendement du sol joue un rôle important dans la médiation de l'incertitude de la concentration Cu. L'influence des différents amendements pour générer des réponses Cu dispersée, du plus grand au moindre impact, est la suivante: l'effet lié à l'UNT> effet lié à CPM> effet lié à la terre DL> effet lié à DLX CPM sol.

Les résultats obtenus ont souligné le rôle des interactions entre les facteurs de variabilité du sol et des amendements appliqués à contrôler le niveau et la dispersion des teneurs en Cu et ont suggéré que la variabilité Cu n'est pas motivée par un paramètre du sol en particulier, mais par des interactions entre eux. Dans tous les cas, l'amendement DLX CPM conduit à la stabilisation (réduction de la variabilité par rapport à la variabilité du pH, DOC et CE) de la concentration de Cu (valeur inférieure de l'écart type) par rapport aux sols non traités(UNT) et aux sols traités par les amendements CPM etDL.

Les résultats généraux obtenus par la méthode montrent que la méthode ANN-MCS stochastique proposée (i) peut être appliquée pour effectuer une analyse de sensibilité des facteurs du sol sur une réponse du sol testée et (ii) peut être utilisée au niveau de la recherche en tant que méthode pour déterminer les paramètres les plus importants à un problème de contamination du sol qui pourraient ensuite servir à élaborer un modèle mécanistique et pour déterminer où les efforts de recherche futurs devraient être ciblés.

3.3.2. Article 7 (*Publié dans Environmental Management Journal*)

Uncertainty analysis of copper phytoavailability in phytoremediated contaminated soils using a neural network and Monte Carlo simulations

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Abstract: Metals in the soil solution are subject to mass transfer out of the system by leaching to groundwaters and plant uptake. Statistical variation in soil environmental conditions and variations in pH, DOC and EC mediated by various soil amendments may combine randomly to affect the metal mobility. Hence, variation in the extent of soil contamination is strongly related to variation in the soil properties and their stochastic combinations. This paper describes a method for the stochastic analysis of the effects of the variation in some selected soil factors (pH, DOC and EC) on the concentration of copper in dwarf bean leaves grown in the laboratory on phytoremediated contaminated soils treated with different amendments. The method is based on a hybrid modeling technique using both artificial neural network (ANN) and Monte Carlo Simulations (MCS). Because the repeated analyses required by MCS are time-consuming, the ANN is employed to predict the copper concentration in dwarf bean leaves in response to stochastic combinations of soil inputs. The input data for the ANN are a set of selected soil parameters generated randomly according to a Gaussian distribution to represent the parameter uncertainties. The output is the copper concentration in the bean leaves. The results obtained by the stochastic ANN-MCS method show that the proposed approach (i) can be applied to perform a sensitivity analysis of soil factors on a given soil response and (ii) can be used as a method to determine the most important parameters in a soil design problem that could then serve to formulate a mechanistic model and to determine where future research efforts should be targeted.

Keywords: Soil contamination, Copper, Bean leaves, Soil factors uncertainty, artificial neural network, Monte Carlo simulation

1. Introduction

Metals in the soil are subject to mass transfer out of the system by leaching to ground water and plant uptake (Alloway, 1995). The concentration of metals in the soil solution is governed by a number of interrelated processes, including inorganic and organic complexation, oxidation/reduction reactions, precipitation/dissolution reactions, and adsorption/desorption reactions (Sauve´ et al., 2000; Weng et al., 2002; Kabata-Pendias and Pendias, 1992; Lexmond, 1980; McBride, 1989; Tyler and Olsson, 2001; Bolan et al., 2003; Chlopecka, 1996 a, b). The ability to predict the concentration of a given metal in the soil solution depends on the accuracy with which the soil inputs such as pH, electrical conductivity (EC) and dissolved organic carbon (DOC) of the soil can be measured (Minasny

and McBratney, 2002). Spatial and temporal variability of soil chemical and physical properties within a field are unavoidable. Many factors can influence the variability of soil parameter measurements, ranging from field sampling technique and soil location, to sample preparation and quality control in the laboratory. If the uncertainty associated with the model output (predictive uncertainty) can be quantified and propagated into the model output, it may provide useful information for many model application purposes (Deutsch, 1994; Gotway and Rutherford, 1994; Srivastava, 1996; Goovaerts, 1999). Laboratory soil tests to investigate the effect of the variability of these properties due to uncertainties are usually time-consuming and laborious. In fact a large number of factors (inputs) involving random fluctuations in time and space are adequately described by stochastic processes. Hence, a rapid modeling tool is needed to perform stochastic analysis (Goovaerts, 2001). Stochastic analysis should result in practical information about the actual importance of potential soil factors (Goovaerts, 2001). The effects of the stochastic variation in soil factors due to uncertainty of the measurements is still lacking for several reasons: (i) the complexity and length of time needed to perform the experiments; (ii) the very high number of experiments to be conducted to obtain statistically significant results; (iii) our lack of knowledge on the statistical distribution of the soil factors; and (iv) the fact that all mathematical predictive models of soil problems have uncertainty associated with them (Deutsch, 1994; Gotway and Rutherford, 1994; Srivastava, 1996; Goovaerts, 1999; Goovaerts, 2001).

It is now widely recognized that mechanistic modeling of soil problems opens the way for stochastic analysis in engineering practice (Deutsch, 1994; Gotway and Rutherford, 1994; Srivastava, 1996; Goovaerts, 1999; Goovaerts, 2001). While deterministic models are useful for exploring the relationship between soil inputs and outputs, it is now up to stochastic methods to expand and generalize the procedures and hence make them applicable to systems large enough to be of interest for soil engineering practices.

Statistical variation in soil environmental conditions and variations related to the uncertainties of pH, DOC and EC combined with various soil amendmentsmay affect trace metal mobility (Goovaerts, 2001; Vanderborght et al., 1997; Pachepsky and Acock, 1998; McKenna, 1998; Bloom and Kentwell, 1999; Broos et al., 1999). Hence variation in the extent of soil contamination is strongly related to the variation in the soil properties. Most studies of the behavior of metals in soils have been carried out under deterministic conditions. Statistical variation in soil pH, DOC and EC, which play a major role in the mobility of metals, suffers from a lack of stochastic (as opposed to deterministic) modeling and analysis. Thus, stochastic modeling of the statistical variation of the soil factors, in particular the soil pH, DOC and EC, is necessary to investigate the stochastic variation induced in the metals' mobility in soils (Goovaerts, 2001).

In the current study we consider the specific case of the prediction of uncertainty of Copper (Cu) toxicity in phytoremediated contaminated soils versus the uncertainties of some a-priori selected soil parameters. Cu toxicity was evaluated by the Cu concentration in bean leaves (BL) grown in the laboratory on the contaminated soils. These soils were treated with four different amendments.

The need for a rational stochastic treatment of uncertainties in Cu concentration in BL analysis is investigated. However, due to the strong dependence of the accuracy on the soil factors, accurate Monte Carlo Simulations (MCS) usually come with a high computational cost. Moreover, stochastic analyses generally require considerably more computation resources and a longer computation time compared to deterministic methods.

The application of stochastic simulation algorithms in the field of soil analysis is a common tool for decision-making processes such as the delineation of contaminated sites (Goovaerts, 2001; Vanderborght et al., 1997; Pachepsky and Acock, 1998; McKenna, 1998; Bloom and Kentwell, 1999; Broos et al., 1999). Several methodologies have been used to account for uncertainty such as

Kalman filtering (Peter, 1979; Ahsam and O'Connor, 1994), first-order analysis (FOA) (Chaubey et al., 1999; Haan and Skaggs, 2003a, 2003b), MCS (Haan and Skaggs, 2003a, 2003b; Ogle et al., 2003; Wang et al., 2005), Latin hypercube sampling (LHS) (Pebesma and Heuvelink, 1999) and generalized likelihood uncertainty estimation (GLUE) (Beven and Binley, 1992; Beven, 1993). Recently, Oporto et al. (2012) applied MCS to identify the cause of soil cadmium contamination. The parameter uncertainty was taken into account with the MC analysis. Different algorithms have been applied by several authors to perform stochastic modeling (Deutsch, 1994; Gotway and Rutherford, 1994; Srivastava, 1996; Goovaerts, 1999). These studies indicated that (i) no simulation algorithm is valid for all cases, and (ii) most of them require the mathematical description of the relationships between soil inputs (properties) and soil outputs which have to be stated a priori in the regression models (Goovaerts, 2001).

Rapid and accurate analysis of the uncertainties involved in the assessment of soil factors by stochastic methods is still lacking compared to deterministic procedures.

In the current work, a hybrid stochastic analysis approach (ANN-MCS) was developed based on ANN modeling and MCS allowing for the prediction of soil output uncertainty based on soil input parameter uncertainty and to examine the predictive uncertainty as a function of model inputs across the full range of parameter space.

ANNs have been widely used in the field of soil science for the prediction of soil hydraulic properties (Schaap and Leij, 1998; Minasny et al., 2004), the generation of digital soil maps (McBratney et al., 2003; Behrens et al., 2005) and the modeling of the behavior of trace metals (Buszewski and Kowalkowski, 2006; Anagu et al., 2009 ; Gandhimathi and Meenambal, 2012). With ANN methods, the relationships between soil inputs (properties) and soil output characteristics do not need to be stated a priori (Anagu et al., 2009; Sarmadian and Taghizadeh Mehrjardi, 2008; Schaap and Leij, 1998). The ANN is trained to find these relations using an iterative calibration process (training phase). It has been reported that ANNs provided superior predictive performance when compared with conventional mathematical methods including multiple linear regression (MLR) models (van der Zee and van Riemsdijk, 1987; Andersen and Christensen, 1988; Streck and Richter, 1997; Römkens and Salomons, 1998; Tiktak et al., 1998; Schaap and Leij, 1998; Elzinga et al., 1999; Sarmadian and Taghizadeh Mehrjardi, 2008).

The ANN-MCS procedure generates random statistical combinations of soil inputs and performs the corresponding stochastic predictions compatible with prescribed statistical distributions, which are assumed to be Gaussian, related to the soil factors. The use of the normal distribution is often defended by invoking the central limit theorem, which states that any distribution will tend to behave like a normal distribution as the values are averaged together mainly for independent variables.

The required input data for the ANN-MCS model are: the mean value and the standard deviation of the pH value, the electrical conductivity (EC), and the dissolved organic carbon (DOC).

The outputs consist of an estimation of the Cu concentration in the bean leaves. The use of an ANN is motivated by the approximate limited experimental data needed to perform a reliable stochastic analysis and the time-consuming repeated analyses required by MCS (Bucher, 1988)

It is shown that the traditional deterministic conception can be easily extended by applying stochastic concepts. The MCS procedure is the key for these developments, as it allows the straightforward use of the currently used deterministic analysis procedure performed by an ANN.

2. Material and methods

The idea here is to train an ANN to provide computationally inexpensive estimates of the Cu concentration in BL, and then to apply MCS using the ANN calculations to predict Cu concentration in dwarf bean leaves (BL) grown on phytoremediated contaminated soils for 10 thousand randomly

generated soil parameter combinations to assess the efficiency of assisted phytosabilisatoon.

The major advantage of a trained ANN over the conventional MLR methods, with the proviso that the predicted results fall within acceptable tolerances, is that reliable results can be produced very rapidly.

The following flowchart describes the different steps and their interdependencies to perform combined ANN-MCS (Fig.1)

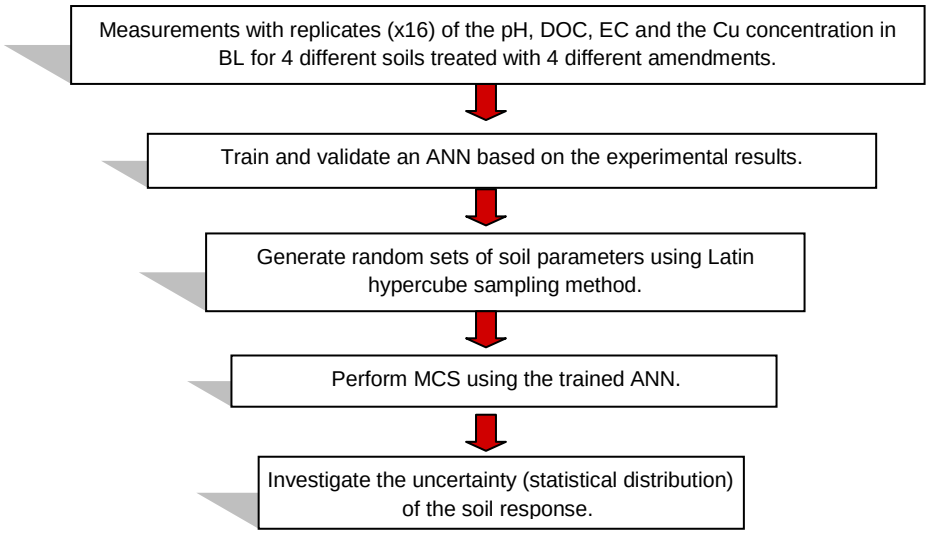


Figure 1. Flowchart of the hybrid stochastic computation approach: ANN-MC.

For an individual case of Cu prediction, the procedure for obtaining the stochastic solution that incorporates soil parameter uncertainties is as follows:

(1) The values of each input variable (pH, DOC and EC) are generated randomly based on their statistical distribution (normal distribution in our case with a mean value and a standard deviation for each parameter);

(2) Deterministic ANN prediction of Cu concentration in BL is performed in response to the random data set generated in step 1;

(3) The above two steps are repeated ten thousand times as part of the MCS;

(4) Finally, the predicted Cu concentrations in BL obtained in step 3 are used to determine the Cu concentration statistical distribution function related to the uncertainty of each input factor (pH, DOC and EC).

2.1. Experiment

2.1.1. Soil sampling and preparation

16 soil samples with 16 replicates each were collected from 16 plots (1x3m) to a depth of 0.25 m from the BIOGECO phytostabilization platform installed on a former wood preservation site located in south-western France, Gironde County (44°43'N; 0°30'O). This site has been contaminated with high concentrations of Cu. The history of the site and its characteristics are detailed in (Mench and Bes, 2009; Bes et al., 2010). Long-term aided phytostabilization experiments are established at the site (Bes et al., 2010). On these 16 plots four different amendments were applied, one per plot (Latin square design) and carefully mixed in the top soil (0-0.30 m) with a stainless steel spade with sixteen replicates: untreated soil (UNT), 0.2% of dolomite limestone (DL), 5% of compost of poultry manure and pine bark (CPM) and a mixture of 0.2% DL along with 5% CPM (DLX CPM). One kilo of each soil was placed in a pot after sieving (2 mm). Four seeds of dwarf beans (*Phaseolus vulgaris*) were sown in each pot and cultivated for 18 days in

controlled conditions (16 h light/8 h darkness regime). The soil moisture was maintained at around 50% of the field water capacity with additions of distilled water after weighing. Then the soil moisture was raised to 80% (11-13 % of air-dried soil mass) at the beginning of the germination stage of the seeds. At the end of the growing period the plants were harvested and the dry weight of BL was determined after drying at 70C°.

The BL were weighed (35-150mg) directly into Savillex Polytetrafluoroethylene PTFE 50mL vessels, 2mL H_2O and 2mL supra-pure 14 M HNO3 were added and the vessels were heated open at 65C° for 2 hours. Then the caps were closed and the containers were left overnight at 65C° (12-14h). After that they were opened, 0.5mL of H_2O_2 (30%) was added to each sample and left open at 75C° for 3 hours. Then 1.5+/-0.5mL of *Fluorhydric Acid* HF (48%) was added to each sample, caps were closed and left at 100C° overnight. The containers were opened and kept at 120C° for 4-5 hours evaporating to dryness, then taken off the heat; 1mL HNO_3 + 5mL H_2O + 0.1mL H_2O_2 were added to each, gently warmed up and after cooling down made up to 50mL. Finally the mineral composition in BL was determined by ICP-MS (Varian 810-MS).

2.1.2. Characterization of soil solution

The soils were watered with distilled water after harvesting the dwarf beans and daily maintained at 80% of field capacity (11-13% of air-dried soil mass) for 2 weeks. Three Rhizon soil-moisture samplers (SMS) from Rhizosphere Research Products (Wageningen, Holland) were inserted after 2 weeks for 24h with a 45^o angle into each potted soil (4x16 soils) to collect soil pore water (30 mL) from each pot. Then dissolved organic carbon (DOC) was analyzed in the soil solution by a Shimadzu© TOC 5000A analyzer. Soil pH and EC were determined in the same soil solution by pH meter and the electrical conductivity (EC) readings.

2.1.3. Selection of soil factors

The mobility of most trace metals in the soil and subsoil depends on the physico-chemical properties of the solid and liquid phases (Fic and Schroter, 1989). The fate and transport of metals in the environment depends on these phases and the interaction between them as trace metals can be found in particulate, colloidal and fully dissolved phases.. Soil parameters, such as pH, organic carbon content, electrical conductivity, iron and manganese oxide content and total metal content are the factors which affect the distribution of copper between different soil fractions (Wilhelmy et al., 1996).

It has been reported that the relationships between the concentrations of metals in plants and a given soil are mainly influenced by the soil properties related to the ion charges (Fisher and Binkley, 2000; Weng et al., 2002; Kerndorff and Schnitzer, 1980). Therefore, in the current preliminary study, the soil inputs for the ANN model were limited to the three measurable factors (pH, DOC and EC) considered to be the most influential on the mobility and availability of metals in the soil.

Kabata-Pendias and Pendias (2000) and Jackson and Miller (2000) reported that the addition of amendments increased the soil pH compared to the untreated soil, leading to a reduction in the mobility and the availability of metals to plants. The second variable used for our work was DOC; Hsu and Lo (2000) noted that the addition of amendment to the soil increased the soil DOC contents compared to that of untreated soil, which led to increasing the plant mass, reducing the bioavailability of metals by forming ligands with the dissolved organic matter. The third parameter was the EC. Du Laing et al. (2008, 2009) and Hatje et al. (2003) found that an increase in conductivity (salinity) increased the mobility of some metals such as cadmium and zinc, thereby increasing the availability and the bioavailability of these metals to the plant.

In the present work, among the various factors identified, the soil inputs were limited to pH, EC and DOC to investigate the influence of the stochastic combination of variation of these parameters due to statistical scatter.

2.1.4. Estimation of the parameters'statistical distribution

4 soil samples were collected from 4 plots treated with 4 different amendments (UNT, CPM, DL and DLX CPM). 16 replications were performed for each measurement, generating 256 (4 soils x 4 amendments x 16 replicates) measured data used to estimate the statistical characteristics of the factors and to train the ANN. The total soil solution concentrations of Cu, pH, DOC and EC were statistically analyzed by (Statistica) to evaluate the Probability Density Function (PDF) of each measured variable and its corresponding characteristics. The Anderson-Darling normality test was performed. The calculated P-Value was greater than 0.05 and the data points follow an almost straight line. The estimation of the different parameters' mean values, their standard deviations and coefficient of variation (COV) is reported in table 1. It can be seen in table 1 that the average concentration and standard deviation vary with the treatment applied.

2.2. Artificial Neural networks

The ANN architecture is composed of an input layer, a certain number of hidden layers and an output layer in forward connections. Each neuron in the input layer represents a single input parameter. These values are directly transmitted to the subsequent neurons of the hidden layers. The neurons of the last layer represent the ANN outputs (Fig. 2).

The output y_i^m of neuron *i* in a layer *m* is calculated by (Johansson et al., 1992; Hagan et al., 1996; Haykin, 1999; Hambli et al., 2006; Hambli, 2010, 2011; Hambli et al., 2011).

$$y_i^m = f\left(v_i^m\right) \quad (1)$$

$$v_i^m = \sum_{j=1}^{L} w_{ji}^{m-1}\, y_j^{m-1} + b_i^m \quad (2)$$

Where y_i^0 are the model inputs, v_i^m are the outputs of the layer *m*, *f* is the activation function, *L* is the number of connections to the previous layer, w_{ji}^{m-1} corresponds to the weights of each connection and b_i^m is the bias, which represents the constant part in the activation function.

Among the activation functions, the sigmoid (logistic) function is the one most usually employed in ANN applications. It is given by (Johansson et al., 1992; Hagan et al., 1996; Haykin, 1999; Hambli et al., 2006; Hambli, 2010, 2011; Hambli et al., 2011):

$$f\left(v_i^m\right) = \frac{1}{1+\exp\left(-\theta v_i^m\right)} \quad (3)$$

where θ is a parameter defining the slope of the function ($\theta = 0.9$).

The training process in the ANN involves presenting a set of examples (input patterns) with known outputs (target output) (Johansson et al., 1992; Hagan et al., 1996; Haykin, 1999; Hambli et al., 2006; Hambli, 2010, 2011; Hambli et al., 2011). The system adjusts the weights of the internal connections to minimize errors between the network output and the target output (Levenberg-Marquardt back-propagation (BP) training algorithm in the current work). The knowledge is represented and stored by the strength (weights) of the connections between the neurons (Johansson et al., 1992; Hagan et al., 1996; Haykin, 1999).

Table 1. Statistical propertiesof the selected soil factors for different soil treatments (PDF: Probability Density Function)

Treatment	Factor	Mean	Standard Deviation	COV (%)	PDF
	pH	**6.87**	**0.092**	**1.340**	**Normal**
UNT	**EC (mS.cm^{-1})**	159.5	15.397	9.653	Normal
	DOC (mg.L^{-1})	347.37	25.35	7.2981	Normal
	pH	6.975	0.055	0.789	Normal
CPM	**EC (mS.cm^{-1})**	161	14.302	8.883	Normal
	DOC (mg.L^{-1})	352.37	23.77	6.746	Normal
	pH	7.05	0.055	0.780	Normal
DL	**EC (mS.cm^{-1})**	162.4	13.352	8.222	Normal
	DOC (mg.L^{-1})	362.54	22.07	6.088	Normal
	pH	7.15	0.041	0.573	Normal
DLX CPM	**EC (mS.cm^{-1})**	163.1	12.552	7.696	Normal
	DOC (mg.L^{-1})	356.7	21.03	5.896	Normal

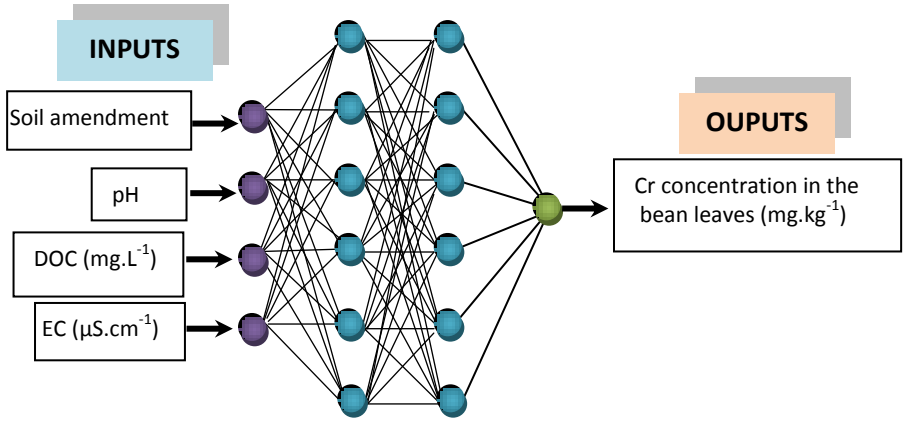


Figure 2. Artificial neural network architecture composed of 4 inputs, two hidden layers and one output layer.

2.2.1. Neural network training

64 measurements were performed; 40 were used for training, 16 samples for testing and 8 samples covering a wide range of the experiments for validation. The testing data were not used for training. The testing data provided cross validation during the ANN training for verification of the network prediction accuracy. The validation data were used to measure the performance of the predictive capability of the ANN after complete training.

In the present work, an in-house ANN program called Neuromod written in Fortran (Hambli, 2010, 2011; Hambli et al., 2011) was applied. The basic ANN configuration employed in this study has a double hidden layer with six neurons in each layer with a learning rate factor $\eta = 0.1$ and momentum coefficient $\alpha = 0.1$ (Fig. 2). The learning rate coefficient η and the momentum term α are two user-defined BP algorithm training parameters that affect the learning procedure of the ANN. The training is sensitive to the choice of these net parameters. The learning rate coefficient, employed during the adjustment of weights (w_{ji}^{m-1}), was used to speed up or slow down the learning process. A larger learning coefficient increases the weight changes, hence large steps are taken towards the global minimum of error level, while smaller learning coefficients increase the number of steps taken to reach the desired error level. Tests performed for

more than two hidden layers and different η and α parameters showed no significant improvement in the obtained results.

2.3. Monte Carlo simulation

For stochastic analysis using MCS, *N* samples of the vector of random soil inputs were generated randomly according to a statistical distribution function. The implementation of the method consisted in the numerical simulation of these samples with an ANN.

As shown in Figure 3, the stochastic modeling concept based on MC sampling can be considered as the direct mapping of the input space onto the output space. The input space represents the random combinations of input factors, each of which follows a probability density function (PDF) representing the statistical uncertainties variability.

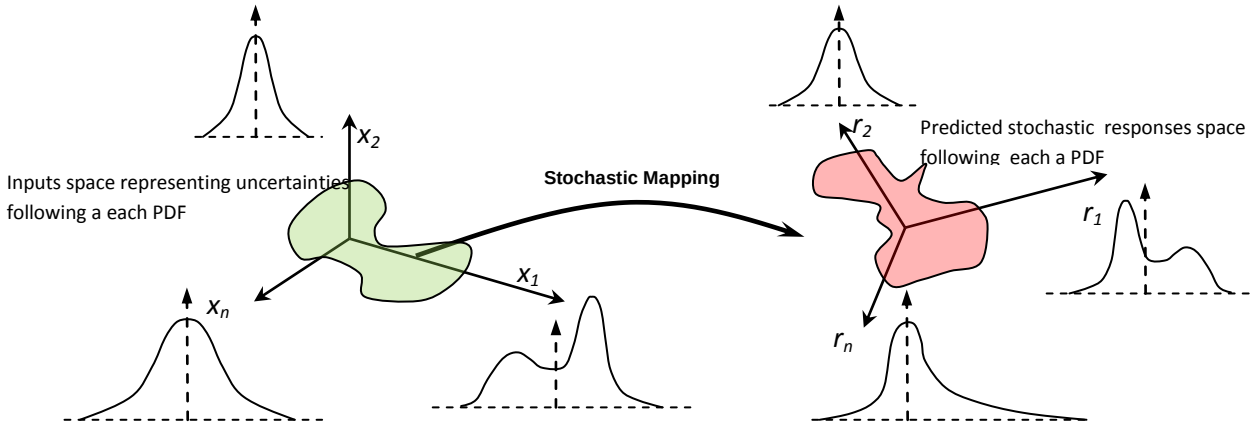


Figure 3. Stochastic modeling concept based on MC sampling considering factor uncertainties consisting of mapping input space (due to uncertainties following probability density functions) onto output space (predicted uncertainties and corresponding probability density functions).

Statistical applications for the study of contaminated sites commonly make use of theoretical distributions such as the normal, log-normal and exponential distributions. The most commonly used distribution model in soil statistics is the normal distribution.

The variables in their basic form may be described as so-called random variables. Typical examples are the soil pH, DOC and EC. Uncertainties in the input parameters naturally result in uncertainties of the output (response). The associated Cu concentration in BL uncertainty is quantified by ANN computation which makes it possible to predict the response probability density functions.

2.3.1. Sampling method for Monte Carlo simulations

In the stochastic analysis of a system, the MCS method is particularly applicable when an analytical solution is not attainable and the experimental procedures are time-consuming. This is mainly the case in soil problems of a complex nature with a large number of basic variables.

Latin hypercube sampling (LHS) was used for the MCS to select random points from the uniformly distributed parameter space. One of the advantages of the LHS method which makes it appropriate for this study is that LHS ensures full coverage over the range of each variable so that all areas of the sample space are represented by the selected input values (McKay et al., 1979; Pebesma and Heuvelink, 1999). The more points that are selected from the parameter space, the more densely the space will be covered and the more reliable the results will be. In the current work, 10,000 random combinations were used for the ANN-MCS analysis.

3. Results

Figure 4 shows the accuracy of predicting BL Cu concentration with the trained ANN for the four different soil treatments. It can be seen that (i) the ANN prediction is in good agreement with the

experimental results, which means that the ANN has been sufficiently trained and (ii) the accuracy of the four soil methods is quite comparable. The R^2 value of the regression is greater than 0.97 and the slope of the regression is close to 1.

To investigate the role of soil parameters'uncertainty, the ANN-MCS model was run, providing 10,000 predictions corresponding to 10,000 random combinations of the uncertain soil parameters. These results were then processed to determine the statistical characteristics of Cu response regarding input uncertainty.

Figure 5 shows the effects of the three soil factors uncertainties on the Cu concentration in BL. The input data were generated randomly according to the normal distribution (Table 1). For an explicit comparison between the different soil treatments and the corresponding data scatter, the mean value and standard deviation were normalized (unit normal distribution: $\mu = 0$ and $\sigma = 1$).

In order to study the influence of each soil parameter, only one parameter was generated randomly for each calculation. For the other factors, the mean values were taken (Table 1).

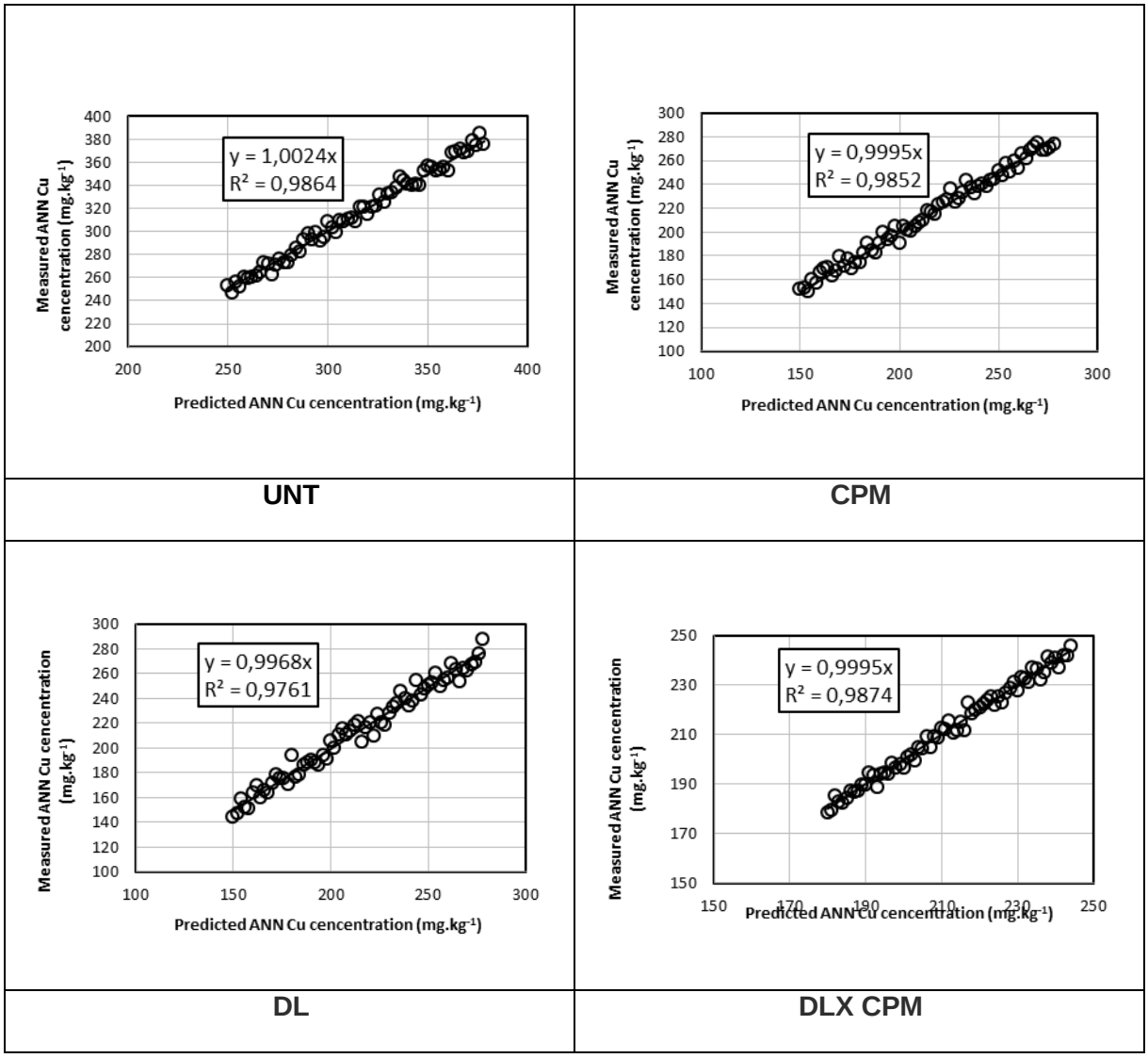


Figure 4. Predicted (ANN) versus Experimental results of Cu concentration in BL Diagrams showing the accuracy of the ANN predicting for different soil treatments.

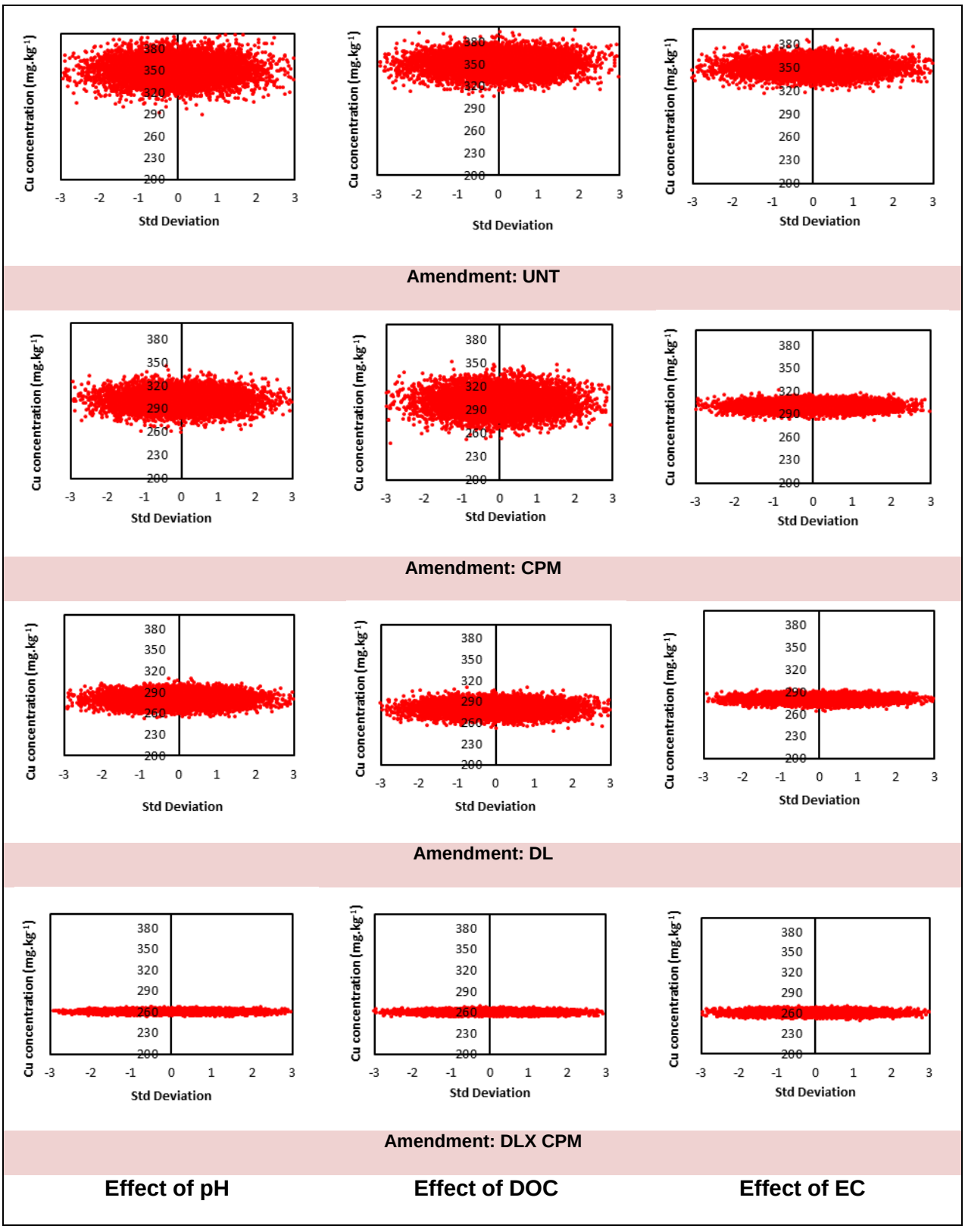


Figure 5. Copper concentration variation versus the statistical variation of soil factors for different amendments.

It can be observed that the scatter is significant due to the uncertainty related to the measurement of the soil parameters. The figure shows the stochastic nature of the predicted Cu concentration in BL generated by the statistical variation of the soil inputs. The measured variability is clearly statistically significant. The scatter plots in Figure 5 indicate that the predicted Cu

concentration in BL for the different treatments follows the normal distribution with varying mean values, standard deviations and coefficients of variation (COV).

The results revealed pronounced differences between the four different soil treatments. The pH measurement uncertainty generated the highest scatter on the Cu concentration in BL. The results indicate clearly that the effect of the pH, DOC and EC uncertainties on the uncertainty of Cu concentration in BL under the influence of the different amendments are as follows, from the greatest impact to the least impact: effect of pH> effect of DOC> effect of EC.

It can be observed that the soil treatment plays a significant role in the output uncertainty. Figure 5 clearly shows that the influence of the different amendments, from the greatest to the least impact, are as follows: effect related to UNT> Effect related to CPM > Effect related to DL soil>Effect related to DLX CPM soil.

4. Discussion

Significant statistical scatter may occur in the Cu concentration in the BL due to local variations in soil factors. Such statistical variability will ultimately result in uncertainties in the predicted Cu in the BL.

Figure 5 indicates that a large scatter of the Cu response reveals an unfavorably high sensitivity to input parameters which are insufficiently known. The Gaussian variation of the pH and DOC generated a significant scatter on the Cu concentration compared to the EC. The EC has a limited (but not negligible) influence on the Cu response. The mobility of trace metals depends on their chemical speciation, which in turn is related to the chemical properties of the soil. Differences in hydrological conditions, temperature and the spatial variability of soil properties contribute to the uncertainty of Cu mobility in soils. Soil parameters such as pH, organic carbon content, iron and manganese oxide content and total metal content affect the mobility of copper among different soil fractions (Fic and Schroter, 1989).

Our results agree with the findings of Sauve´ et al. (2000). The authors reported that the availability of the soil metals to the plants depends on two factors: (i) the physico–chemical parameters such as pH and ionic strength, (ii) the presence of several sorbents in the soil such as organic matter.

The distribution of soil pH is controlled by several environmental factors, such as acid deposition, parent rock, altitude, soil types, land use types, vegetation cover, human activities, etc. (Boruvka et al., 2007). The soil pH data may vary severely within a short horizontal distance. Soils are not homogenous and the pH can vary spatially considerably from one spot in the field to another. It also varies with depth (Trangmar et al., 1985; Warrick et al., 1986; Mulla and McBratney, 2000). Soil variability also occurs across soil series and soil units and may be large or small depending on different soil forming factors. Al-Omran et al. (2004) reported that measurement of the central tendency of experimental variability of pH revealed that it follows a normal distribution.

Many authors have studied the effect of pH variability on the mobility of metals in the soil. Brown et al. (1997) studied the effect of high pH on metal mobility, and found that an increase in soil pH led to an increase in the mobility of most metals in the soil. The authors concluded that these metals formed mobile fulvic acid metal complexes. When the soil pH increases, the solubility of fulvic acids increases, thus promoting the formation of more fulvic acid complexes. In addition, Kabata-Pendias and Pendias (1992), Lexmond (1980), McBride (1989), and Tyler and Olsson (2001) reported that the bioavailability and toxicity of Cu in the soil is related to changes that can occur in soil pH. Where they found that the availability increased in acidic soil relative to calcareous soil, this increase was due to the increase in the concentration of Cu^{+2} (free Copper) in the soil solution which generally presented the available form of Cu to the living organisms (plants, microorganisms). McLaren and Crawford (1973) explained that increasing the soil pH leads to an increase in the binding of Cu to soil constituents, and therefore a decrease in the mobility and availability of soil Cu.

Kumpiene et al. (2008) reported that the stability, mobility and availability of Cu in soil are strongly pH dependent. The mobility of Cu increases with decreasing pH. Borg and Johansson (1989) and Gallagher et al. (2001) reported that Cu is largely retained in the soil. Its mobility in the soil increased with the decrease in pH. The addition of carbonates, phosphates and clays to soils can keep Cu mobility in soil low by chemisorption (Kabata-Pendias and Pendias, 2000), the application of coal fly ash to soil increases soil pH and the amount of carbonates which have the capacity to control Cu mobility in soil (Jackson and Miller, 2000).

However, Ciccu et al. (2003) found that increasing soil pH to above 8 by the application of coal fly ash (15% by weight) and red mud–gypsum (15% by weight) showed less than 50% and 10% efficiency, respectively, while a combination of these amendments even increased Cu leaching by 170% over the untreated soil. The mobility of Cu usually decreases at slightly alkaline pH but can increase in highly alkaline conditions (>10) due to the formation of OH_ complexes (van der Sloot et al., 1997). Finally, the effect of acidity on the mobility and availability of metals (Cu) in soil is unclear because its effect is the result of the interaction of many factors in the soil, but the result is that with only slight variations in soil pH, we obtained significant effects on the availability of Cu in the studied soil.

As part of active organic carbon, DOC plays a certain role in the mobility of contaminants between the particulate and dissolved phases (Huang et al., 1998), due to the net negative charge of DOC at typical soil pH, quick circulation, ready availability for uptake and sensitivity to environmental changes. The variability in the quantity and the quality of DOC, of humic and fulvic acids, and the ability of these compounds to bind metals are the factors that determine the availability and mobility of metals in the soil. In addition, DOC has the ability to form stable, soluble complexes with trace metals (e.g. Cd, Cu, Ni, Pb, Zn), and plays an important role in the adsorption of soil trace metals (Dawson et al., 1978; Qualls et al., 1991; Kalbitz et al., 2000). Castilho et al. (1993) pointed out that the concentration of metals in the soil solution increased with an increase in the concentration of the DOC and that it was not affected by a change in pH. They also concluded that Cu is associated with DOC. They performed cation exchange experiments with nine membrane filtered topsoil filtration extracts and found that Cu was associated with low molecular weight complexes. Thirty to seventy percent of the copper was bound to fast-dissociating complexes which are the complexes formed with low molecular weight DOC.

The mobility and availability of metals in soil is also related to the binding of the metals to the organic soil fraction. This binding depends on the total number of both strong and weak binding sites, which is related to the number of functional groups. The total acidity i.e. the number of carboxylic acids and phenol hydroxyl groups is often used as a measurement of the binding capacity (Gerke et al., 1994). Balasoiu et al. (2001) reported that the organic matter of soils can bind significant amounts of Cu by forming an OM-bound Cu fraction which presents about 96% of the total Cu in CCA-contaminated soil.

Howell and Gawthorne (1987) discussed the important role of organic matter in the retention and behavior of Cu. They reported both humic and fulvic acids as two ligands for copper (II), with binding to fulvic acids being more soluble than to humic acids. They also reported simple aliphatic acids, amino acids and aromatic acids as chelating agents for copper (II). Igloria et al. (1997) included the possibility of the formation of complexes between trace metals and organic matter colloids in solutions, as well as those attached to soil surfaces. Hsu and Lo (2000) reported that the affinity of Cu for organic matter has twofold consequences: in the presence of dissolved OM, Cu mobility increases as Cu–HA and Cu–FA complexes are formed, whereas non soluble high molecular weight organic acids can retain significant concentrations of Cu in soil upon soil acidification (Chirenje and Ma, 1999). Sposito (1986), on the other hand, claimed that the effect of these colloids might retard metal transport. Ma and Rao (1997) suggested that the chemical speciation of the metals determined metal behavior and mobility in the environment and finally their availability to plants.

In general, EC can be influenced by a number of different soil properties, including clay content and soil water content (Kachanoski et al., 1990; Morgan et al., 2001). Aimrun et al, 2009 reported that the variability of soil within a field can generate variability in the EC measurement. Du Laing et al. (2008), (2009) and Hatje et al. (2003) found that an increase in conductivity (salinity) led to a increase in the mobility of some metals such as Cadmium and Zinc, thereby increasing the availability and the bioavailability of these metals to the plant. Amrhein et al. (1993) studied the effect of the changes in soil salinity and the quantity of colloidal fraction in the soil on metal mobility. They reported that the enhancement of metal mobility leachate from such soils was associated with soil salinity and colloidal particles. Under high salinity, there was little evidence of colloid-assisted transport. Under low salinity, dispersion and mobilization of colloidal-sized particles associated with trace metals such as Cu, Pb, and Ni occurred. They concluded that the mobility of these trace metals and colloidal particles increased when the salinity decreased.

To assess the prediction accuracy, a comparison between the measured variability characteristics and the predicted ones shown in Figure 5 is given in table 2.

Treatment		Mean value ($mg.kg^{-1}$)			Standard Deviation ($mg.kg^{-1}$)			COV (%)		
	Factor effect	ANN-MC	Measured	Error (%)	ANN-MC	Measured	Error (%)	ANN-MC	Measured	Error (%)
UNT	pH	352.645	343.450	-2.677	18.333	17.013	-7.759	5.199	4.954	-4.955
	DOC ($mg.L^{-1}$)	350.710	340.145	-3.106	16.100	17.150	6.122	4.591	5.042	8.944
	EC ($mS.cm^{-1}$)	351.870	338.450	-3.965	11.130	10.125	-9.926	3.163	2.992	-5.730
CPM	pH	299.200	317.740	5.835	17.500	16.989	-3.008	5.849	5.347	-9.392
	DOC ($mg.L^{-1}$)	298.100	311.580	4.326	19.050	18.179	-4.791	6.390	5.834	-9.522
	EC ($mS.cm^{-1}$)	299.200	314.450	4.850	8.560	8.188	-4.546	2.861	2.604	-9.876
DL	pH	271.120	256.950	-5.515	14.670	15.267	3.910	5.411	5.942	8.931
	DOC ($mg.L^{-1}$)	269.330	254.230	-5.940	15.780	16.374	3.628	5.859	6.441	9.031
	EC ($mS.cm^{-1}$)	271.230	257.870	-5.181	6.300	6.570	4.110	2.323	2.548	8.823
DLX CPM	pH	252.820	243.130	-3.986	3.400	3.620	6.077	1.345	1.489	9.666
	DOC ($mg.L^{-1}$)	250.920	267.540	6.212	5.430	5.870	7.496	2.164	2.194	1.370
	EC ($mS.cm^{-1}$)	252.780	278.460	9.222	3.650	4.030	9.429	1.444	1.447	0.224

Table 2. Comparison between predicted and measured statistical properties (mean value, standard deviation and coefficient of variation) related to the selected soil factors (pH, DOC and EC) for four different soil treatments (UNT, CPM, DL and DLX CPM).

It can be seen that the predicted and measured results are in good agreement, with an error ranging from -9.93 % to 9.67 %. This suggests that the good agreement between model predictions and observations is not driven by a particular soil parameter but by interactions among parameters. The results indicate that the uncertainty of the Cu concentration in BL may be dependent on the applied amendment. In our case, we found that pH is the factor which generates the highest uncertainty on the soil response for the UNT, CPM and DL treatments. Surprisingly, the DLX CPM caused a net decrease in pH uncertainty (low value of standard deviation and COV) (Fig. 6).

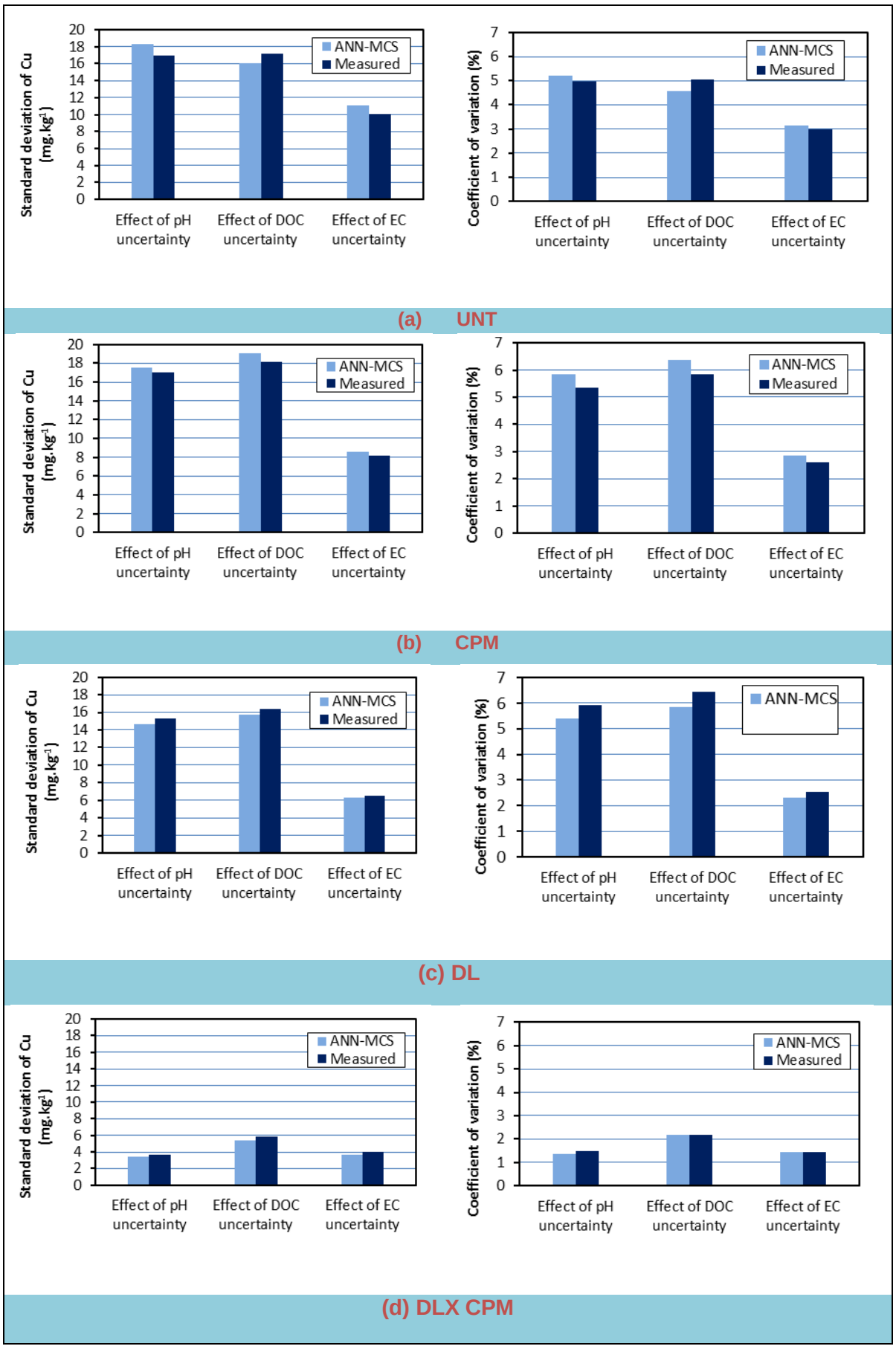
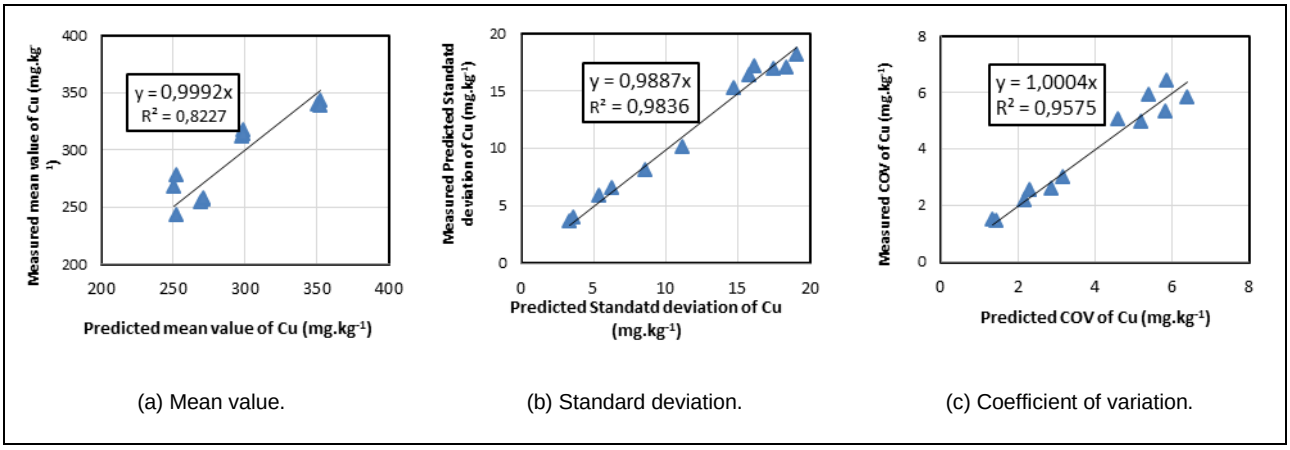


Figure 6. Predicted and measured standard deviations and coefficients of variation related to the uncertainties of pH, DOC and EC for four amendments (UNT, CPM, DL and DLX CPM).

Comparisons between the predicted and measured data (table 2) are plotted in Figure 7. The prediction/measurement correlation gives a R^2 of 0.8227 with a slope of 0.9992 for the mean value of Cu, a R^2 of 0.9836 with a slope of 0.9887 for the standard deviation and a R^2 of 0.9575 with a slope of 1.0004 for the COV (Fig. 7).



(a) Mean value. (b) Standard deviation. (c) Coefficient of variation.

Figure 7. Comparison between measured and ANN-MCS predicted mean value (a), standard deviation (b) and coefficient of variation (COV) (c).

The close agreement in mean, standard deviation and COV indicates the similarity in measured and predicted variability of Cu concentration in BL. This indicates that the proposed ANN-MCS model is acceptable for predicting uncertainties (statistical characteristics and distribution) as a consequence of the uncertainties of the soil inputs. This is useful in decision making when based on model output. A hybrid ANN-MCS modeling approach has been developed to perform stochastic analysis of Cu concentration in BL. The model takes into account the inherent uncertainty of the soil factors. The method is based on the presentation of stochastic soil parameter combinations as a series of random variables to predict Cu concentration in BL. By using a certain number of MCS, this makes it possible to investigate the sensitivity of the response related to each single soil factor and their combinations. If the uncertainty associated with the model output (predictive uncertainty) can be quantified and propagated into the model output, it may provide useful information for many model application purposes. For example, the average metal concentration in plants predicted by the ANN-MCS model can serve as an indicator to help assess metal contamination and to choose adequate remediation practices. The soil factors governing the behavior of Cu mobility are always subject to a certain amount of scatter. Thus for an enhanced and reliable prediction such uncertainties should be taken into account when investigating the relationship between the soil inputs and outputs. The total time required to develop and run the ANN-MCS is dependent upon: the time required to generate the training and test data used; the time taken to train a number of ANN models in order to select the appropriate complexity; and the time required running the ANN for the required number of MC samples. The majority of time is spent on generating the training data. In this study, the time required for the ANN prediction of 10,000 input combinations was approximately 25 sec.

Despite its good performance, the proposed hybrid ANN-MCS method suffers from a number of limitations.

First, ANN-MCS is not based on a physical description of the problem, which limits the physical understanding of these uncertainties. Moreover there are various types of uncertainties

related to other soil/plant factors, and the entire spectrum of uncertainties is not yet known. In this preliminary work, only the uncertainty of three selected a-priori soil parameters was studied. It would be of interest to study the effect of these uncertainties on the Cu concentration in BL. In this way, an overall view would be obtained of the effect of all uncertainties. However, such a comprehensive approach will be time consuming and expensive due to the complexity and the time needed to generate large experimental patterns to train the ANN. Second, the available statistical information is mainly restricted to the mean value, the standard deviation, upper and lower fractal values or upper and lower bounds which characterize the normal distribution. Other probability density functions such as log-normal and exponential distributions may be applied instead of the normal distribution. However, with the ANN-MCS approach, whatever the statistical information available, it can be used in relation with a specific sampling procedure for MCS. Third, it should be noted that the proposed ANN approach does not take into account all the possible factors which may influence soil toxicity. The primary aim was to illustrate the potential of the ANN method in its ability for rapid and accurate prediction of soil toxicity. The ANN model can be extended by including additional factors and their combinations to capture complex Cu behavior. Nevertheless, ANNs have a number of significant benefits that make them a powerful, rapid and practical tool for solving many soil engineering problems. Third, there are no general guidelines which can help in the design of ANN-MCS modeling by a non-specialist. There is a need to develop a user-friendly interface that can be used by a non-specialist.

In this paper, an ANN-based MCS approach for stochastic analysis of Cu concentration in BL given a limited number of soil inputs has been presented. The focus of the proposed approach was not on achieving an in-depth investigation of metal contamination, but rather on (i) presenting the concept of combined rapid ANN-MCS modeling and (ii) presenting a preliminary predicted statistical characteristic to highlight the potential of the method.

In conclusion, the current preliminary study showed that ANN in conjunction with MCS may represent a viable approach for simulating stochastic processes in various soil engineering fields. This approach is particularly powerful in cases where various properties of the process cannot be identified or specified precisely. Contrary to traditional methods, a model specification is not required. Moreover ANN possesses a higher intrinsic complexity than traditional simulation models.

Before its large-scale application, however, further investigations are needed by performing extensive experimental validation based on several experiments including additional factors to capture complex soil behavior.

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Conclusion générale et perspectives

1) Conclusion générale

Le risque inhérent à un contaminant est intimement lié à sa possibilité de transfert vers l'organisme cible ou vers d'autres compartiments comme la nappe phréatique. Concernant les éléments traces, les mécanismes contrôlant leurs transferts du sol aux organismes vivants (notamment les plantes) et leurs effets sur les écosystèmes doivent être compris afin de choisir une stratégie de remédiation adaptée.

Par ailleurs, si les techniques classiques de décontamination sont très efficaces pour les contaminants organiques, elles s'avèrent lourdes, onéreuses, voire inadaptées pour les éléments traces non biodégradables. Depuis quelques années, les biotechnologies ont montré que l'utilisation de plantes supérieures peut aider à stabiliser ou éliminer les concentrations excessives des éléments traces des sols contaminés.

Dans ce travail, la stratégie expérimentale consiste à comparer l'efficacité de différentes options de phytoremédiation en situation sur un sol ancien et sur un technosol tous deux polycontaminés.

1) Le confinement de la contamination est réalisé en évitant son transfert par une couvert végétal approprié (phytostabilisation) ; cette technologie est accompagnée parfois par des agents fixants comme les amendements organiques ou inorganiques ou un mélange des deux, qui peuvent aider à stabiliser les contaminants dans le sol;

2) La décontamination des sols est obtenue en favorisant le développement d'une population végétale accumulant les éléments traces (phytoextraction); cette technologie utilise des plantes métallicoles accumulatrices.

La première étude réalisée sur le sol naturel contaminé en Cu, Cr, As et Zn a été conduite à la suite d'un cycle de quatre années de culture de peupliers mycorhizés et de saules. Dans ce contexte de phytostabilisation, trois types d'amendements ont été testés pour augmenter *l'efficacité de la*

phytostabilisation : le compost (OM), la dolomie (DL) ou le mélange composte + dolomie (OMDL). Les résultats que nous avons obtenus sur les échantillons de sol prélevés sur ce site ont montré que la matière organique (OM) a été le meilleur amendement. On se base sur le test d'exposition conduit par la culture de haricots nains cultivés sur les échantillons de sols en pot au laboratoire. Les sols des parcelles où est incorporé l'amendement OM montrent une diminution des concentrations foliaires de Cu, Cr, Zn et As dans les haricots. Par contre seules les minéralomasses de Cr et As ont été réduites. La concentration de Cu dans l'eau interstitielle du sol (SPW) a augmenté pour les sols amendés avec OM et OMDL alors qu'elle a légèrement diminué dans le sol avec DL. L'ajout de l'OM et DL, seuls ou en combinaison, a généré une diminution de la fraction disponible du Cu dans le sol et de l'intensité d'exposition du sol.

La deuxième étude réalisée au cours de cette thèse a porté sur le même site avec pour objectif d'évaluer l'*efficacité de la phytoextraction assistée* pour remédier (par élimination) les fortes concentrations du Cu. Les parcelles sélectionnées pour cette étude sont caractérisées par des paramètres physico-chimiques identiques mais avec la présence de concentrations croissantes de cuivre total dans les sols, ces concentrations sont en augmentation comme suit: 163, 268, 382, 518, 753, 1170 mg Cu / kg respectivement pour les parcelles traitées avec des amendements, et 832 mg Cu / kg pour la parcelle non traitée. En complément une parcelle témoin non contaminée de la même terrasse alluviale a été utilisée comme sol de contrôle. Un cycle de culture de deux ans a été appliqué sur ces parcelles, en alternant plusieurs espèces de tournesol et de tabac, plantes accumulatrices. L'échantillonnage de ces sols a été réalisé au cours de ces deux années. La mesure des concentrations dans les eaux porales (SPW) en laboratoire confirme un résultat connu dans la littérature ; les concentrations totales en Cu dissous augmentent linéairement avec la concentration totale de Cu dans le sol et avec elle l'exposition. La deuxième année cette concentration a diminuée : la phytoextraction a été très efficace car la rotation des cultures de tournesol et de tabac a diminué significativement les concentrations totales dissoutes en Cu mais aussi en Zn, Cr et As (des SPW). En même temps l'intensité de l'exposition du sol pour tous les éléments traces potentiellement toxiques (PTTE) a diminué de façon significative après deux années de rotation des cultures comme le montrent les tests de phytodisponibilité réalisés en mesurant la concentration dans les feuilles de haricots nains: la masse minérale du Cu augmente ainsi linéairement avec les concentrations totales du Cu dans le sol. En résumé, on a trouvé une forte corrélation entre les concentrations croissantes du Cu dans le sol, les fractions dissoutes et la phytodisponibilité pour le haricot. Il s'agit là d'un résultat très positif en faveur de la technique de phytoextraction du Cu notamment.

On s'est ensuite intéressé à l'efficacité de la phytostabiliation assistée, dans un technosol polycontaminé en PTTE. Il s'agit de sols déstructurés tels qu'on les rencontre sur les friches industrielles ou minières. Cette étude a été réalisée sur une friche industrielle instrumentée, le technosol de Châteauneuf (site ADEME). Nous avons travaillé sur les neuf parcelles lysimétriques. Sur ce type de sol qui n'a aucune activité pédogénétique, l'apport d'amendement est un des premiers paramètres à tester. En effet, la mauvaise qualité du substrat est un facteur limitant pour la phytostabilisation des sites métallurgiques et miniers. Outre la contamination polymétallique et les pH souvent extrêmes (ici pH très basique), les technosols sont déséquilibrés en éléments nutritifs, pauvres en matière organique et souvent très drainants. Dans ces conditions, il est important de favoriser la reconstruction d'un sol apte à supporter un couvert végétal et à limiter les pertes d'eau. La stratégie consiste à initier l'activité pédologique grâce à deux types d'amendements organiques : le premier est un amendement organique frais (bois raméal fragmenté (RCW) qui a pour but d'accélérer l'implantation de la pédofaune (biomasse vivante). Le RCW est susceptible à terme de favoriser les processus d'humification, de réduire les pertes d'eau, et finalement, d'accélérer la reconstruction d'un sol équilibré et biologiquement actif. L'autre est un amendement organique mature (compost : CSS) efficace dans les sols en fonctionnement.

Nos résultats montent que l'ajout de la matière organique sous une forme ou l'autre, a eu d'emblée un effet positif sur le technosol. RCW a été l'amendement le plus efficace pour réduire les concentrations dissoutes de tous les éléments traces étudiés dans la solution du sol (SPW), tandis que le CSS a été efficace pour quelques éléments uniquement (Mo, Cr et Co). L'intensité de l'exposition au Zn évaluée par DGT n'était pas significativement modifiée par l'ajout de RCW et CSS, tandis que l'intensité de l'exposition au Cr évaluée par DGT a été significativement diminuée après l'addition RCW par rapport au sol traité avec CSS et le sol sans amendement. Les deux amendements RCW et CSS ont la capacité de diminuer la concentration foliaire de Mo, Zn, Cr, As et Co dans les feuilles de haricots nains qui servent de test de phytodisponibilité en laboratoire sur les sols, alors que les deux amendements augmentent la concentration foliaire de Cu. De plus l'amendement RCW a été plus efficace que CSS pour réduire la minéralomasse de Mo, Cr et Co dans les feuilles de haricots. Ces résultats montrent que l'addition des amendements organiques, notamment RCW ont un effet positif sur le technosol. Ils ont en effet diminué les concentrations dissoutes des éléments traces, et donc réduit la phytodisponibilité de ces éléments. Nous arrivons à la conclusion ***qu'il est possible d'amender un technosol pour obtenir une couverture végétale tout en stabilisant une polycontamination sévère vis-à-vis de la nappe phréatique.***

Avec sa reconstruction et le retour de l'activité biologique le technosol retrouve un cycle d'humification et se pose alors la question du rôle de la matière organique vis-à-vis des contaminants : l'humification ne va-t-elle pas favoriser la mobilité des contaminants ? Pour répondre à cette question, nous avons repris les mêmes cycles et les mêmes parcelles lysimétriques du site de l'ADEME de Châteauneuf. La reconstruction d'un sol équilibré et biologiquement actif va générer un cycle d'humification. La question scientifique qui est adressée concerne la capacité des substances humiques liées aux deux types d'amendements (matières organiques fraiches, CSS et matures, RCW) à complexer les éléments traces et à modifier leurs spéciation et leur mobilité. A ce stade précoce de mise en route de l'activité pédologique, nous avons trouvé que l'addition du CSS au sol a amélioré ses caractéristiques chimiques, comme attendu. L'amendement organique mature a naturellement augmenté de façon significative ($p < 0{,}001$) le pourcentage de C_{org} associé aux fractions HA par rapport aux sols amendés par le RCW et les sols non amendés (NE). Les sols amendés avec CSS ont montré le taux d'E4/E6 le plus élevé et le taux d'E2/E3 le plus faible des acides fulviques extraits. Les sols amendés en CSS possèdent donc les plus petites fractions organiques avec un faible degré de condensation des composés aromatiques et un caractère hydrophile par rapport aux autres sols. En regardant l'association des éléments traces avec les acides humiques et fulviques, on trouve que le Zn et l'As sont plus abondants dans la fraction acide fulvique que dans la fraction acide humique. Au contraire Pb, Cu et Mo ont été trouvés plus associés aux acides humiques qu'aux acides fulviques dans les sols traités et non traités, ce qui peut expliquer la différence de la mobilité et de la disponibilité du Zn et As par rapport au Pb, Cu et Mo.

En complément aux mesures expérimentales, la modélisation par réseaux de neurones a ensuite cherché à déterminer quel était le facteur le plus déterminant du sol sur la phytodisponibilité des éléments traces dans le sol. Cette phytodisponibilité est normalement le résultat de la mobilité et la disponibilité de ces éléments dans le milieu transférant du sol (solution de sol). Dans un premier temps on a utilisé le modèle des réseaux de neurones artificiels (ANN) comme un outil rapide et précis pour la prédiction de la concentration de Cr dans les haricots nains cultivés en laboratoire sur un sol contaminé en Cu, Cr, Zn et As phytorémédié. Il s'agit d'étudier l'effet des différents types d'amendements, pH du sol et DOC du sol sur la phytodisponibilité du Cr. L'autre objectif a été de montrer le facteur le plus influent sur ces concentrations dans les plantes. Nous avons trouvé que la matière organique (CPM) a été l'un des meilleurs amendements utilisé sur ce site et sous ces conditions expérimentales, car elle a réduit les concentrations de Cr dans les feuilles de haricots nains (BL) plus que les autres amendements. Les interactions entre les facteurs du sol sous la forme de

surfaces de réponse ont été analysées. Les résultats ont montré que le pH du sol a un effet plus fort que le COD dans le même sol et sous les mêmes conditions.

L'application des réseaux de neurones artificiels dans les domaines de la modélisation du comportement des sols sont souvent résolus directement, ce qui signifie que la réponse du sol (sorties) est déterminée par rapport à un ensemble d'entrées. Inversement, on peut être intéressé par l'évaluation d'un ensemble de facteurs d'entrées du sol conduisant à des réponses (sorties) du sol (cible). Ce travail a été notre deuxième application des ANN sur le même sol contaminé. Nous avons développé et testé un modèle ANN inverse pour la prédiction du traitement du sol optimal pour réduire la phytotoxicité du cuivre à partir d'entrées sélectionnées. Nos résultats ont montré que la méthode proposée peut réussir à identifier le meilleur traitement de sol à partir des propriétés du sol (entrées). L'amendement CPM a la capacité de réduire la toxicité du Cu quelles que soient les valeurs de pH, EC et DOC dans le sol, alors que les amendements DL et DLX CPM ne sont pas en mesure de réduire la toxicité cible du Cu en-dessous de 200 $mg.kg^{-1}$ quelles que soient les valeurs de pH, EC et DOC.

Dans les sols, on sait que la variabilité spatiale et temporelle des paramètres physiques et chimiques au sein d'une même parcelle est inévitable. Plusieurs facteurs peuvent influer sur la variabilité de mesure des paramètres du sol et sur la précision des mesures, allant de la technique de l'échantillonnage de terrain à la préparation des échantillons et à la qualité des mesures effectuées dans le laboratoire.

Dans notre travail la variation statistique des paramètres du sol comme le pH, DOC et CE générée par l'addition de différents amendements au sol dans le cadre de la phytostabilisation assistée peut se combiner stochastiquement pour influer sur la mobilité et la phytodisponibilité des éléments traces. Nous avons essayé dans notre travail de décrire une méthode pour l'analyse stochastique d'effet de la variation de certains facteurs du sol comme le pH, DOC et CE sur la concentration de cuivre dans les feuilles de haricots nains cultivés en laboratoire sur les sols contaminés, afin d'étudier l'influence de la phytoremédiation sur la toxicité du sol contaminé. La méthode est basée sur une technique de modélisation hybride utilisant un réseau de neurones artificiels (ANN) et des simulations de Monte Carlo (MCS). Nos essais ont montré que la méthode ANN-MCS stochastique proposée **(i)** peut être appliquée pour effectuer une analyse de sensibilité des facteurs du sol sur une réponse du sol testée. En effet, dans notre travail, on a trouvé que la variabilité (dispersion statistique naturelle) des concentrations du Cu dans le haricot a été sensible

dans un ordre hiérarchique à la variabilité (dispersion statistique) du pH, suivie par la variabilité du DOC. Cependant on a remarqué que la sensibilité à la conductivité électrique EC a été faible dans les sols. **(ii)** La méthode ANN-MCS peut être utilisée au niveau de la recherche en tant que méthode pour déterminer les paramètres les plus importants à un problème de contamination du sol qui pourrat ensuite servir à élaborer un modèle mécanistique et pour déterminer où les efforts de recherche futurs devraient être ciblés.

Pour résumer, l'ensemble des résultats obtenus résultants des diverses expérimentations et modélisations a permis d'obtenir des données robustes sur la capacité de la phytostabilisation et de la phytoextraction, assistés par des amendements, à remédier et restaurer les sols naturels ou les technosols fortement contaminés en éléments traces métalliques. En particulier, on a prouvé que l'application d'ANN sur les sols contaminés est très efficace. Ce modèle (ANN) a permis de prédire d'une façon très fiable et très rapide les réponses d'un sol contaminé, de mieux analyser les effets de chaque facteur sur la phyto disponibilité des métaux aux plantes. Enfin, le modèle ANN a été combiné avec une procédure de simulation stochastique de type Monte Carlo pour étudier les effets de la variation statistique des paramètres du sol générés, modulés par l'addition de différents amendements au sol sur la variabilité des concentrations des métaux dans les sols contaminés.

2) Perspectives

Les travaux effectués au cours de cette thèse souffrent de quelques limitations qui alimentent les perspectives envisagées. Celles-ci concernent deux aspects: (i) les perspectives concernant la partie expérimentale, (ii) les perspectives concernant la partie modélisation.

(i) Les perspectives de la partie expérimentale

Dans ce travail nous avons contribué à l'étude et à l'évaluation de l'efficacité de deux techniques de phytoremediation à restaurer deux sites d'études différents. Ces deux sites sont:

1) le sol naturel de Bordeaux : Sur ce site on a appliqué la phytostabilisation et la phytoextraction pour restaurer ce site fortement contaminé en Cu. Les résultats obtenus ont montré que les deux techniques sont efficaces pour réduire les concentrations excessives des éléments traces. Cependant la phytoextraction a été appliquée seulement sur deux ans. Par conséquent nous manquions des données de phytoextraction sur plusieurs années pour pouvoir valider ou invalider cette option de phytomanagement.

Par contre, dans un sol contaminé et en présence de plantes, il ne faut pas négliger l'effet de l'exsudat racinaire sur la spéciation des éléments traces et finalement sur leurs fixations ou élimination du sol. Les études futures devraient porter sur la rhizosphère.

2) Le technosol de Rive du Gier : Sur ce site on a appliqué également la phytostabilisation assistée afin d'évaluer son effet sur l'écodynamique des éléments traces dans le sol. Les résultats présentés dans ce travail ont été obtenus après un an de phytostabilisation, au début de l'addition de la matière organique fraiche (RCW) et mature (CSS) au sol. Nous conseillons donc de refaire des expériences à long terme pour surveiller l'effet de l'évaluation de l'état de la matière organique ajoutée au sol. En particulier, le bois raméal fragmenté n'a pas eu le temps de se dégrader complètement. Par contre l'addition de la matière organique dans le sol et spécialement la matière organique fraîche comme RCW, favorise le développement des microorganismes du sol, lesquels ont certainement un effet sur la spéciation et la phytodisponibilité des éléments traces dans le sol.

(ii) Les perspectives de la partie modélisation

Les réseaux neuronaux artificiels (ANN) ont été largement utilisés comme des outils de prédiction rapide et précis dans de nombreuses applications. Notre étude bibliographique a montré que l'application des ANN dans le domaine des sols est assez limitée malgré le très grand potentiel que les ANN offrent pour l'analyse des résultats. Typiquement, les travaux publiés utilisant les ANN dans les sols considèrent souvent le problème direct, ce qui signifie que les modèles ANN ont été construits pour prédire les réponses du sol (sorties) en fonctions de facteurs d'entrées mesurables. Cependant, on peut être intéressé par le problème inverse à savoir, l'estimation des valeurs de facteurs d'entrées générant des réponses cibles. Cette approche est désignée par le terme « ingénierie inverse ».

Dans ce travail on a montré le potentiel des ANN en développant à la fois les modélisations directs et inverses comme outils rapides et précis pour (i) la prédiction de la concentration de Cr, Cu, AS et Zn dans les haricots sous l'effet de différents types d'amendements, pH, DOC et EC du sol, (ii) la classification des facteurs du plus influant vers le moins influant sur ces concentrations et (iii) effectuer une étude de stochastique pour analyser la variabilité des réponses et (iv) identifier l'amendement optimal pour un sol.

Ces résultats sont intéressants ; cependant une modélisation plus complète doit tenir compte d'autres paramètres ou variables du sol comme par exemple la capacité d'échange cationique (CEC), la granulométrie du sol et l'humidité du sol. Nous recommandons également d'effectuer plus de

répliquats pour avoir plus de fiabilité et précision à notre modèle. Nous recommandons également dans ce type du problème environnemental d'analyser l'effet de la variabilité de ces facteurs testés et mesurés dans les sols, sur la sensibilité de l'exposition aux concentrations des éléments traces dans les sols contaminés.

Enfin, notons que les modèles ANN développés dans le cadre de cette thèse peuvent être étendus et complétés pour :

- Modéliser le comportement d'un site à plus grande échelle : ceci nécessite de penser et préparer les essais selon un plan d'expérience spécifique.
- Développer des interfaces graphiques conviviales sous l'environnement Windows pour une utilisation simple et immédiate par un non-spécialiste : Ceci peut être réalisé rapidement à l'aide d'un langage de programmation objet comme le C++ et Visual.Net.
- Modéliser l'évolution temporelle des réponses : Ceci nécessite de disposer/effectuer d'essais espacés dans le temps afin que l'ANN intègre le temps comme variable d'entrée lors de la phase d'apprentissage.

Les résultats des modèles ANN présentés confortent ces perspectives qui vont dans le sens d'un transfert de technologie entre la recherche sur les sols et les applications d'ingénierie opérationnelle.

Nour HATTAB

Ecodynamique des éléments traces et caractérisation de l'exposition desols contaminés phytorémédiés: expérimentation et modélisation par les Réseaux de Neurones Artificiels

Les sols contaminés par les éléments traces potentiellement toxiques (PTTE) ont souvent des conséquences graves pour les écosystèmes terrestres. Plusieurs options de phytoremediaction ont été développées pour remédier les sols contaminés ; cependantl'efficacité et la capacité de ces techniques à réduire les concentrations excessives des éléments traces ou leur (phyto) disponibilité dans les sols contaminés doivent être évaluées Le présent travail s'est intéressé à étudier l'efficacité de deux options de de phytorémédiation, la phytostabilisation et la phytoextraction assistées par des amendements organiques et minéraux, à remédier les fortes concentrations de PTTE dans un sol naturel et dans un technosol contaminés. Les concentrations totales des éléments traces dissous ont été déterminées dans l'eau interstitielle du sol. L'intensité de l'exposition du sol a été évaluée par des capteurs DGT (gradient de diffusion dans les couches minces). Le phytodisponibilité des PTTE a été caractérisée par des tests de germination avec des haricots nains cultivés sur les sols contaminés pour lesquels les concentrations foliaires en éléments traces ont été déterminées. Ensuite un modèle de réseau de neurones artificiels a été appliqué pour comprendre les facteurs les plus pertinents sur la variabilité de la phytodisponibilité des PTTE.

Les deux options ont étécapables de réduire les concentrations ou la phytodisponibilité des PTTE en présence des amendements. Les réseaux de neurones artificiels ont été très efficaces pour prédire les résultats manquants et pour déterminer les paramètres de contrôle de la variabilité de la phytodisponibilité des PTTE à partir des paramètres du sol.

Mots clés : sols contaminés, phytoremediation, amendement, éléments traces potentiellement toxiques, réseaux de neurones artificiels

Ecodynamics of trace elements (ET) and characterization of the exposure of contaminated phytoremediated soils: experimentation and modeling by Artificial Neural Networks

Soils contaminated with potentially toxic trace elements (PTTE) often have serious consequences for terrestrial ecosystems. Several phytoremediaction have been developped to reclaim contaminated soils; however the efficiency and capacity of these techniques to reduce excessive concentrations of trace elements or their (phyto) availability in contaminated soils have to be assessed.

The present work is focused on studying the effectiveness of two phyoremediation options such as phytostabilisation and phytoextraction assisted by organic and inorganic amendments to remediatethe high concentrations of PTTE in contaminated natural soils and technosoils. Total PTTE concentrations were determined in soil pore water (SPW) sampled by Rhizon soil moisture samplers. The soil exposure intensity was assessed by DGT (diffusive gradient in thin films) probes. The PTTE phytoavailability was characterized by growing dwarf beans on potted soils and analyzing their foliar PTTE concentrations. Then a model of artificial neural network was applied to understand the factors most relevant for the variability on the phytoavailability of trace elements.

Both options were found to be able to reduce the concentrations or phytoavailability of PTTE in the presence of amendments. The artificial neural network has been very effective to predict missing results and to determine the control parameters of the variability of the PTTE phytoavailoability from the soil parameters.

Keywords: contaminated soils, phytoremediation, amendment, potentially toxic trace elements, artificial neural networks.

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